VOLUME 49 APRIL, 1959 NUMBER 4

Phytopathology

An International Journal

UNIVERSITY OF MICHIGAN
A. 2 1959
SCIENCE LIBRARY

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONTENTS

Vancomycin, a potential agent for plant disease prevention
P. P. Mehta, David Gottlieb, and Dwight Powell 177

White tip disease of rice. II. Seed treatment studies E. H. Todd and John G. Atkins 184

White tip disease of rice. III. Yield tests and varietal resistance John G. Atkins and E. H. Todd 189

Methods for microscopic detection of Aphanomyces euteiches and Rhizoctonia solani and for isolation of Rhizoctonia solani associated with plant debris M. G. Boosalis and A. L. Scharen 192

Multiple virus disease resistance in a strain of Capsicum annuum
A. A. Cook and C. W. Anderson 198

Locus, time, and mode of action of western X-little cherry virus in sweet cherry trees in Oregon
Leo E. Jones 202

The effect on various cultures of Erysiphe graminis f. sp. hordei of the genes in barley that condition resistance to culture CR3 J. G. Moseman and C. W. Schaller 207

Variation in Phytophthora palmivora isolated from cacao and rubber R. G. Orellana 210

Combination reactions of three small-grain viruses on wheat
Sardar B. Lal and Webster H. Sill, Jr. 214

Virus increase in seedling roots C. E. Yarwood 220

Studies on the control of citrus canker with streptomycin
G. Rangaswami, Rao Rama Rao, and A. R. Lakshmanan 224

Report and abstracts of the 1958 annual meeting of the Northeastern Division of the American Phytopathological Society 227

Phytopathological Notes

Fusaric acid in banana plants infected with Fusarium oxysporum f. cubense O. T. Page 230

The distribution on Nicotiana glutinosa leaves of lesions produced by tobacco mosaic virus
Irving Rappaport and Sam G. Wildman 231

Use of Cellophane tape for mounting and photographing Phytopathogenic fungi
Edward E. Butler and Margery P. Manns 231

Book listings 232

Charter members and presidents of APS (photographs) 233

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

MILLER "658" Fungicide

(Formerly "Crag* 658")

*T.M.U.C.C.

One of the "top yielding" fungicides in 1958 tests. Controls diseases on potatoes, tomatoes, cucurbits, peanuts, citrus, avocados, azaleas and turf, including such problems as Late Blight, Early Blight and Downy Mildew. Encouraging results were received in 1958 on Gray Leaf Spot and Bacterial Leaf Spot.

Chemical and Physical Properties

ACTIVE INGREDIENTS:

The copper-zinc-chromate complex:

$15\ CuO,\ 10\ ZnO,\ 6\ CrO_3,\ 25\ H_2O$ 95%

INERT INGREDIENT 5%

Active ingredients expressed in terms of metal content:

Copper	29.6%
Zinc	20.4%
Chromium	9.7%
Total	59.7%

Miller "658" Fungicide is a Berthollide or non-Daltonian compound. This fungicide is a greenish-yellow, free-flowing powder of very low water solubility, and extremely fine particle size. The bulk density of this material is 15 lbs. per cubic foot. It is readily suspended in water without the addition of wetting or dispersing agents and may easily be formulated as a dust by dilution with an inert material, such as talc, pyrophyllite, or clay. Spray or dust deposits of Miller "658" Fungicide on foliage are resistant to weathering and the compound is chemically stable both in storage and on foliage.

Write today for Miller Technical Bulletin #29

MILLER
CHEMICAL AND FERTILIZER CORP.
BALTIMORE 15, MARYLAND

VANCOMYCIN, A POTENTIAL AGENT FOR PLANT DISEASE PREVENTION[1]

P. P. Mehta,[2] David Gottlieb, and Dwight Powell

SUMMARY

Vancomycin inhibits the growth of many phytopathogenic bacteria and is very readily absorbed by the leaves and roots of plants. The antibiotic is transported both upward to the growing point and downward to the root, but tends to accumulate in leaves in preference to stems. It can be absorbed by roots against a concentration gradient and is rapidly translocated to the crown. Vancomycin readily moves into young leaves and roots from seeds treated with the antibiotic. When aqueous solutions of the compound are applied to soil, it accumulates in young plants, though some of the antibiotic is inactivated by soil. No toxic symptoms have been observed at concentrations of 1000 μg/ml.

Numerous agents protect plants from disease when applied to the foliage or stems. Their action is primarily topical, preventing the germination of spores on the surface of a plant; some materials also eradicate local infections. Since these chemicals are not usually translocated within the plant, they cannot inhibit growth of the pathogen once the inner tissues are infected. Recent research for new fungicides and bactericides has been directed to synthesis, or to isolation from natural sources, of chemicals that will act systemically, protecting plants at a distance from the site of application. This search currently also involves the study of the systemic action of various antibiotics, and a number of such materials have been shown to be absorbed and translocated in plants (2, 3, 4, 5, 6, 8, 9, 11, 15, 16).

Our studies with the antibiotic vancomycin indicate that this compound possesses many properties that might make feasible its use as a systemic agent for the control of some bacterial diseases.

Vancomycin, an antibacterial agent produced by *Streptomyces orientalis* McCormick et al, was first isolated and characterized by M. H. McCormick and coworkers (13). Further studies on its biological activity have been reported by Ziegler, Wolfe, and McGuire (18). It is a white, water-soluble complex amphoteric molecule with a molecular weight of 3300. Its activity is not depressed in the presence of various salts, amino acids, or vitamins, or in a pH range of 6.5–8.0. Vancomycin has been reported to be active primarily against gram-positive bacteria. It is primarily a bactericidal agent, unlike many other antibiotics, which tend to be bacteristatic at low concentrations. Furthermore, bacteria do not readily develop resistance to this agent, even after serial transfers (18).

METHODS.—The vancomycin used in these experiments, obtained through the courtesy of Eli Lilly and Co., was 80% pure. The following plants were used in various phases of the studies: Bonny Best tomatoes, pepper (Allig-Ill. F5), Florida Market eggplant, Straight Eight cucumber, Dixie Queen watermelon, Little Marvel pea, bush Lima bean, and Turkish tobacco. Seeds were planted in pots containing a sterilized 3:1 mixture of black prairie loam and sand. When an intact root system was needed, vermiculite was used instead of soil. A DeVilbiss atomizer connected to a compressed air line was used to spray the antibiotic onto the plant parts. In other experiments, the roots or leaves were immersed or floated in solutions of the antibiotic for various periods. Unless stated otherwise, all treatments were in triplicate.

To determine the presence of the antibiotic, leaves, stems, or roots were individually washed in running water and then in 3 changes of fresh tap water, blotted dry, wrapped in paper towels, and frozen overnight at −34.5°C. After the plants were allowed to thaw, the juices were obtained by squeezing the samples in either a hand garlic press or a Carver Press at 10,000 psi. The plant juices were assayed by a disc assay method, similar to that described by Loo et al (12) except that a brewer's-yeast–thioglycollate medium was used (17). Lyophilized spores of *Bacillus subtilis* Cohn emend. Prazmowski were suspended in a 1% phosphate buffer at pH 7.0 and the volume adjusted to give a concentration of 4.3×10^7 spores per ml. One ml of suspension was added to each 100 ml of assay medium when the temperature was 45°C. Plates were poured immediately and stored at 3°C until used. Concentration of antibiotic in plant parts was expressed in μg/ml of plant juice. These values were obtained by comparison with a standard curve (concentration vs. zone size), using the Eli Lilly vancomycin as a standard. All assays were made in triplicate. The sensitivity of various bacteria to vancomycin was determined by the agar-plate dilution method, with Penassay Seed Agar. Paper chromatography of the antibiotic was according to the procedure described by Amman and Gottlieb (1).

RESULTS.—*Antibacterial spectrum.*—Contrary to reports that vancomycin is effective primarily against gram-positive bacteria, it also inhibited many gram-negative plant-pathogenic bacteria. The growth of 13

[1] Accepted for publication October 17, 1958.

Portion of a thesis submitted to the Graduate College, University of Illinois, in partial fulfillment of the requirements for the Ph.D degree.

[2] Assistant Plant Pathologist to the Government of Bihar, P.O. Sabour, District Bhagalpur, Bihar, India; formerly Graduate Assistant, Department of Plant Pathology, University of Illinois.

of the 29 phytopathogenic bacteria tested was suppressed at concentrations of 10 μg/ml or less (Table 1). *Corynebacterium tritici* (Hutchinson) Burkholder was most sensitive, being inhibited at less than 0.1 μg/ml. *Xanthomonas campestris* (Pammel) Dowson, *Pseudomonas phaseolicola* (Burkholder) Dowson, and *P. syringae* van Hall were inhibited at less than 1.0 μg/ml, and 6 other species were inhibited at 1.5 μg/ml. The sensitivity of bacteria to the antibiotic varied with both genus and species. The genus *Pseudomonas* was relatively resistant: only 2 of the 10 species tested were inhibited at less than 10 μg/ml; the other 6 required more than 500 μg/ml. Members of *Xanthomonas* were more sensitive: only 2 of the 10 species tested required more than 50 μg/ml for inhibition; 4 were inhibited at 1.5 μg/ml and 4 at 10–50 μg/ml. The 3 species of *Corynebacterium* were all very sensitive, whereas *Erwinia* and *Agrobacterium* varied with the species. Because of the efficacy of vancomycin for inhibiting some of the phytopathogenic bacteria *in vitro*, studies were made of its absorption and translocation in plants.

TABLE 1.—*Antibacterial spectrum of vancomycin*

Test organisms[a]	Minimum inhibitory concentration (μg/ml at 48 hr.)
Bacillus subtilis Cohn emend. Prazmowski	0.4
Pseudomonas phaseolicola (Burkholder) Dowson L.I.	0.7
Pseudomonas syringae van Hall, L.I.	0.5
Pseudomonas tabaci (Wolf & Foster) Stevens, ATCC-11527	>500.0
Pseudomonas aptata (Brown & Jamieson) Stevens, ATCC-10205	10.0
Pseudomonas apii Jagger ATCC-9654	>500.0
Pseudomonas marginalis (Brown) Stevens, ATCC-10844	>100.0
Pseudomonas atrofaciens (McCulloch) Stevens, ATCC-9004	>500.0
Pseudomonas cornafaciens (Elliot) Stevens ATCC-9005	>500.0
Pseudomonas solanacearum E.F. Sm. ATCC-10692	>500.0
Pseudomonas pisi Sackett, ATCC-11043	>500.0
Xanthomonas campestris (Pammel) Dowson L.I.	0.5
Xanthomonas vesicatoria (Doidge) Dowson L.I.	1.5
Xanthomonas pruni (E.F. Sm.) Dowson L.I.	10.0
Xanthomonas pruni (E.F. Sm.) Dowson ATCC-10017	1.5
Xanthomonas carotae (Kendrick) Dowson ATCC-10547	>25.0
Xanthomonas phaseoli (E.F. Sm.) Dowson ATCC-10199	1.5
Xanthomonas beticola (E.F. Sm. et al.) Savulescu ATCC-11677	>25.0
Xanthomonas cajani Kulkarni et al. ATCC-11639	>500.0
Xanthomonas stewartii (E.F. Sm.) Dowson	>100.0
Xanthomonas cassise Kulkarni et al. ATCC-11638	>25.0
Erwinia amylovora (Burril) Winslow et al. L.I.	2.5
Erwinia carotovora (Jones) Holland L.I.	>50.0
Erwinia atroseptica (van Hall) Jennison ATCC-7403	>100.0
Erwinia aroideae (Townsend) Holland ATCC-7404	>100.0
Corynebacterium michiganensis (E.F. Smith) Jensen ATCC-10202	1.5
Corynebacterium flaccumfaciens (Hedges) Dowson ATCC-7392	1.5
Corynebacterium tritici (Hutchinson) Burkholder ATCC-11402	<0.1
Agrobacterium tumefaciens (Smith and Townsend) Conn. ATCC-11157	>500.0
Agrobacterium rhizogenes (Riker et al) Conn. ATCC-11323	1.5

[a] L.I. = local isolate; ATCC = American Type Culture Collection

Stability and filterability.—Vancomycin is a relatively stable compound. It withstands 100°C at a pH near neutrality for at least 1 hour without detectable loss of activity. Twenty-minute autoclaving at 115° and 121° resulted in respective activity losses of only 7 and 10%. Lowering the pH of the solution tended to increase heat lability, with the greatest decrease at the lowest pH used, 2.5. Antibiotics are generally used in low concentrations for experimental studies, and solutions of these materials must often be sterilized by filtration. Some filters are very surface-active, adsorbing the active agent. Relatively little vancomycin was removed by this process in various experiments: 2.8–13.2% of the active material was lost by passage through a Selas micro-porous porcelain candle filter (FD), and none of the antibiotic was adsorbed during passage through sintered glass (UF) filters.

Absorption of vancomycin by plants.—Vancomycin was absorbed from the surface of leaves sprayed with the antibiotic (Table 2). Applied at 50 μg/ml to tobacco, Lima bean, tomato, and cucumber, the substance could not be detected inside the leaves, but it was detectable at an application level of 100 μg/ml or above. Absorption was rapid, and the antibiotic could be demonstrated in the plant within 3 hours. The higher the concentration of antibiotic applied, the greater the amount of antibiotic found in leaves during this period, when concentrations in the leaves were highest. Vancomycin sprays of 100 and 200 μg/ml were inactivated in tomato and tobacco within 48 hours. In Lima bean treated with 100 μg/ml, the antibiotic could not be detected at 24 hours, but the 200 μg/ml treatment was detected at 120, though not at 240 hours. Cucumber retained high levels of vancomycin for at least 240 hours, even at these relatively low rates of application. All 4 plants, when sprayed with 500 and 1000 μg/ml contained active material for at least 120 hours, and, except for beans, some antibiotic remained in the tissues for at least 240 hours. Cucumber leaves absorbed the antibiotic most readily. Sometimes the assays did not indicate a consistent decrease of antibiotic in the leaves with time, but the results nevertheless indicate a general trend of inactivation.

Vancomycin was sprayed in a graded series of concentrations on upper or lower leaf surfaces of the first fully expanded trifoliate leaf of bean plants. Differ-

ences between absorption from upper and lower leaf surfaces were not statistically significant ($P < .05$). Spraying both surfaces resulted in a statistically significant increase in the antibiotic content of leaves over spraying either side alone.

Tobacco plants were different from bean plants. The lower surfaces of leaves absorbed more antibiotic than did the upper surfaces. Another factor that influences rate of absorption is the time of the day of spraying. Among 4 applications, at 10 A.M., 4 P.M., 10 P.M., and 4 A.M., only the 10 P.M. showed a statistically significant ($P > 0.05$) greater absorption than the other times—2–3 times as great as the next greatest accumulation, whether the spray was applied to the upper or lower surfaces of the leaves.

Excised leaves and roots can also absorb vancomycin. Leaves of Lima bean and tobacco were floated 6 hours on solutions of the antibiotic in a series of concentrations between 25 and 1000 μg/ml. Excised roots of the plants were immersed 6 hours in similar solutions. The graph in Fig. 1 shows the uptake of antibiotic by both leaves and roots, and the linear increase in accumulation with increased concentration of the solutions. Deviation from the linear was not statistically significant ($P < .05$) except for the tobacco leaf. Variations within the leaves and roots were negligible. No loss of chlorophyll or other symptoms of phytotoxicity were observed, even in leaves exposed to 1000 μg/ml.

Adding various compounds to increase the absorption of vancomycin was not very effective. One of the twin primary leaves of Lima bean was sprayed with a solution of vancomycin alone, and the other with the same concentration plus various additives. The additive alone was sprayed on the first trifoliate leaf. The antibiotic content of the leaves was not increased by the following additives in the spray: 1% glycerol, 2% ethylene glycol, 2% diethylene glycol. Increases were very slight with 2% propylene glycol or 1% methylcellosolve. Glycerol additives, tried also in sprays on tobacco, gave no increase in absorption. Materials in greater variety were tested by floating excised leaves of Lima bean and tobacco on solutions containing the antibiotic: Tween 80, sesame oil, peanut oil, Triton B-1956, sorbitol, sucrose, mannitol, and starch, besides the additives already listed. Again no marked increases in vancomycin in the leaves were attributable to the additives.

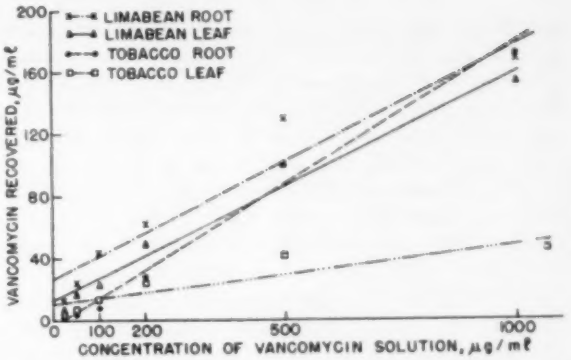


Fig. 1. Absorption of vancomycin by excised leaves and roots.

Roots of entire plants that had been grown in nutrient solution with a vermiculite base and transferred to aqueous solutions of vancomycin absorbed the antibiotic efficiently. Within 3 hours after pepper roots were placed in solutions of the antibiotic at concentrations of 25, 50, 100, and 500 μg/ml, the roots contained 3.6, 16.0, 11.0, and 99 μg/ml of root juice.

Translocation of vancomycin in plants.—Vancomycin was translocated toward both the apical portion and the roots of plants. Sixty-day-old cucumber, Lima bean, pepper, tobacco, and tomato plants were used in these translocation experiments. The middle leaf on each plant was sprayed with a 1% solution of vancomycin while the other plant parts were protected with plastic covers. Control plants were sprayed with

TABLE 2.—*Concentration of vancomycin in leaf juice at various intervals after spraying with the antibiotic*

Plant	Concentration[b]	μg/ml[a] after various periods (hours)						
		3	6	12	24	48	120	240
Tobacco	100	2.1	1.2	0.0	0.0	0.0	0.0	0.0
	200	4.8	2.8	1.3	0.0	0.0	0.0	0.0
	500	33.5	1.8	1.3	1.8	5.6	2.3	3.3
	1000	39.0	9.0	2.6	4.5	9.0	4.6	3.2
Lima bean	100	1.4	1.2	1.3	0.0	0.0	0.0	0.0
	200	2.2	1.5	1.8	2.3	2.0	1.3	0.0
	500	7.7	1.6	2.2	9.5	11.0	4.3	0.0
	1000	56.0	15.0	25.0	22.0	11.0	23.5	0.0
Tomato	100	0.0	4.6	1.2	0.0	0.0	0.0	0.0
	200	3.5	3.1	1.5	2.6	0.0	2.9	0.0
	500	17.5	5.6	7.5	19.5	26.0	14.5	1.7
	1000	10.5	20.0	13.0	18.5	29.5	5.2	6.2
Cucumber	100		2.7	1.1	2.1	2.4	1.8	13.0
	200		4.7	3.4	8.2	7.1	3.4	28.0
	500		19.0	22.5	13.0	37.5	12.5	37.0
	1000		91.0	58.0	48.0	100.0	39.0	98.0

[a] Averages of 3 leaves.

[b] Control solutions containing no antibiotic were also used under the same conditions. No antibiotic was found in plants sprayed with these solutions.

water. Duplicate plants were taken for analysis 3, 6, 12, 24, and 48 hours after treatment. Leaves and stem sections progressively nearer the apical and basal regions, as well as roots, were assayed. The movement of the antibiotic is very rapid, reaching both apical shoot and root within 3 hours. Representative data are given for tomato in Table 3. Similar data were obtained for the other plants. Generally, concentrations of the antibiotic in leaves and stems are greater below the sprayed leaf than above. Vancomycin tended to accumulate in the leaves in preference to the stem, which apparently functions as a channel for movement of the antibiotic. Analyses of leaves and stem portions at increasing distance from the sprayed leaf showed a correspondingly decreasing content of vancomycin. Vancomycin could be detected in the roots of all 5 test plants, though occasionally only in trace amounts. Since only 1 leaf was sprayed, low root concentrations would be expected. Maximum absorption and movement usually occurred within 24 hours of spraying. Vancomycin is more readily absorbed and translocated in cucumber, Lima bean, and tomato than in pepper and tobacco. Within 24 hours, the maximum recovery of vancomycin from the crown below the site of application in cucumber, tomato, Lima bean, tobacco, and pepper was, respectively, 195, 195, 170, 68, and 70 μg/ml of plant juice. Maximum recovery from roots, in the same order, was 3.0, 16.5, 11.5, 6.0, and 2.5 μg/ml. The time of optimum concentrations varied for the different species.

To prove that the antibiotic in the treated plants was indeed vancomycin, a study was made on the movement of vancomycin on paper chromatographs. Juices from sprayed plants were placed on sheets of filter paper alongside solutions of vancomycin and developed in the usual manner. There was no significant difference between the behavior of authentic samples of vancomycin and the antibiotic obtained from the roots, stems, and leaves of the 7 types of plants. Thus, the identity of the antibiotic within the plant is not in question, and one can assume only that it was absorbed from the sprayed leaves. Typical values are presented in Table 4.

TABLE 3.—*Distribution of vancomycin within the tomato plant at various intervals after single-leaf spray*

	μg/ml[a] after various periods (hours)				
Plant parts	3	6	12	24	48
Leaf	2.1	4.0	3.1	11.0	2.4
Stem	3.3	42.0	1.5	1.7	0.0
Leaf	11.5	23.0	5.4	8.4	5.4
Stem	5.6	1.1	3.6	1.0	1.5
Leaf	3.8	7.4	3.5	7.0	10.0
Sprayed Leaf	20.0	33.0	64.0	350.0[b]	66.0
Leaf	32.0	67.0	43.0	33.0	23.5
Stem	7.4	2.7	19.5	3.3	4.8
Leaf	105.0	195.0	165.0	35.0	39.0
Leaf	18.0	8.7	87.0	51.0	50.0
Stem	3.3	1.0	3.8	1.9	7.8
Leaf	130.0	13.5	80.0	170.0	10.0
Roots	16.5	7.0	5.6	3.3	4.5

[a] Average of 2 plants.
[b] This figure appears inordinately high.

TABLE 4.—*Rf values of vancomycin and of the antibiotic in juice obtained from treated plants in different irrigating solvents*

Solvent system	Vancomycin	Tomato	Pepper
n-butanol:acetic acid:water (2:1:1)	0.04	0.04	0.05
n-butanol:pyridine:water (1:0.6:1)	0.45	0.45	0.42
3% ammonium chloride	0.92	0.90	0.89
50% hydrous acetone	0.90	0.86	0.90
water saturated with *n*-butanol	0.91	0.94	0.90

When the middle leaf of the first trifoliate leaves of Lima bean was kept submerged in a solution of vancomycin of 200 μg/ml, movement into other leaves was undetectable even though the material was being absorbed during the entire period. A concentration of 6–9 μg/ml accumulated in the side leaves only after 120 hours. Submerging all 3 of the trifoliate leaves was more efficient, and concentrations of 10 and 6 μg/ml were found in the next higher and lower leaves, respectively, after 24 hours of soaking. Injecting high concentrations of antibiotic into the plant did not appear promising. Within 6 hours of treatment, even when solutions of 1.0 mg/ml were used, only 2.6 μg/ml were detected in the first leaf above the point of injection, and 10.5 μg/ml in the first leaf below. Some movement, both up and down, occurs when cut ends of cucumber petioles are placed in solutions of 200 μg/ml. After 240 hours, the next upper leaf contained 3.0 μg/ml, and the lower one 2.9 μg/ml.

The movement of vancomycin from root to shoot is very efficient. Roots of tobacco, eggplant, pepper, pea, Lima bean, tomato, and cucumber were immersed in aqueous solutions varying in concentration from 5 to 500 μg/ml. Assays of plant parts were made at intervals after treatment of 3–120 hours, and in a few cases up to 360 hours. Table 5 presents a sample of the results obtained. Solutions of the antibiotic as low as 10 μg/ml furnished enough material to be detected in leaves of bean and tomato; solutions of 25 μg/ml were sufficient for the other plants. In most cases the concentration gradient was from the root to the top of the plant. Again it is apparent that relatively little of the antibiotic concentrates in the stem. For example, in the pea at the 120-hour assay, the concentrations of antibiotic in leaves and stems, respectively, for the different solutions of vancomycin were: 6.4 and 0.0 μg for the 25-μg/ml solution; 3.5 and 0.0 μg for 50 μg/ml; 37.0 and 16.0 μg for 100 μg/ml; and 75.0 and 40 μg for 250 μg/ml. The great accumulations in the plant without phytotoxicity is surprising. Occasionally there was more antibiotic in the leaves than in the root; in the 120-hour assay, tobacco plants contained 210 μg/ml in the juice of leaves, and only 97 μg/ml in the roots.

Studies with tobacco, eggplant, pepper, pea, and tomato showed that the concentration of the antibiotic in the plant generally increased as sprays of greater

TABLE 5.—*Distribution of vancomycin in Lima bean plants with roots immersed in a solution of the antibiotic for various periods*

Concentration used (μg/ml)	Part	μg/ml[a] after various periods (hours)				
		24	48	120	240	360
10	Leaf	7.0	6.4	6.4	8.7	0.0
	Stem	0.0	0.0	0.0	0.0	0.0
	Root	55.0	40.0	40.0	60.0	40.0
25	Leaf	87.0	22.0	22.0	22.0	16.0
	Stem	2.1	0.0	0.0	0.0	0.0
	Root	165.0	165.0	99.0	83.0	75.0
50	Leaf	30.0	30.0	40.0	40.0	30.0
	Stem	6.4	3.5	6.4	7.0	4.7
	Root	220.0	165.0	125.0	290.0	165.0
100	Leaf	75.0	75.0	75.0	60.0	75.0
	Stem	30.0	22.0	16.0	11.0	12.0
	Root	505.0	350.0	505.0	220.0	380.0
250	Leaf	75.0	165.0	220.0	75.0	99.0
	Stem	22.0	40.0	30.0	30.0	40.0
	Root	1000.0	690.0	690.0	690.0	380.0

[a] No vancomycin could be recovered from plants whose roots were immersed in the control solutions, which contained no antibiotic.

concentrations were applied. For example, leaves of tobacco, treated with sprays of 25, 50, 100, and 500 μg/ml contained, respectively, 14, 25, 78, and 190 μg/ml plant juice at 120 hours. The respective stems contained 2.0, 3.5, 11.0, and 125 μg/ml.

Plants whose root systems were immersed in solutions of vancomycin accumulated it very rapidly. Roots in a 100 μg/ml solution of antibiotic for 24 hours accumulated vancomycin, in μg/ml plant juice, as follows: tobacco, 49; eggplant, 26; pepper, 9; pea, 75; Lima bean, 505; tomato, 37; and cucumber, 75. Concentration in the leaves, in the same order, was 61, 12, 9, 33, 75, 22, and 12. High root concentrations built up very rapidly. Within 3 hours in solutions of 25, 50, 100, and 500 μg/ml the respective concentrations in roots were 3, 5, 30, and 195 μg/ml of juice in tobacco, and 4, 20, 44, and 97 μg/ml in eggplant. Results with pepper were similar. The compound is absorbed so readily by some species, such as Lima bean, that the root accumulates vancomycin against a concentration gradient (Table 5). In solutions of 10, 25, 50, 100, and 250 μg/ml, roots at 24 hours contained 55, 165, 220, 505, and 1000 μg/ml, respectively. With most plants at most concentrations, the active compound in the leaf increases with the period of root immersion, but more noticeably with plants whose roots absorb the substance slowly. In pepper, for example, roots immersed in a 100-μg/ml solution and assayed at 3, 6, 12, 24, and 48 hours had respective concentrations of 0.0, 2.5, 9.5, 8.8, and 14 μg/ml. Lima bean, on the other hand, accumulated maximum concentrations in the leaves within 24 hours. Immersion of the roots of a plant in solutions of vancomycin is an efficient way to produce high concentrations of the antibiotic in the tissues without any apparent toxic effects.

Seed treatment.—Seeds of tomato, cucumber, watermelon, pea, Lima bean, and pepper were soaked 16 hours in aqueous solutions of the antibiotic, washed in running water, and planted in sterilized soil or vermiculite. There were no significant differences between treated plants and controls in germination percentages, and young plants that grew from the treated seeds showed no toxic symptoms.

The antibiotic can be absorbed by the seed and transported to the young plant (Table 6). Seeds treated with solutions of vancomycin in concentrations of 2–500 μg/ml for 0, 6, 12, and 24 hours were washed in running water and allowed to germinate on moist filter paper in a Petri dish until the primary unexpanded leaves and roots were well formed. Vancomycin was found in the roots and shoots of all 3 test

TABLE 6.—*Distribution of vancomycin in plants after soaking the seed for various periods*

Soak concentrations used (μg/ml)		μg/ml after various soaking periods (hours)							
		0		6		12		24	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Tomato	25	0.0	0.0	0.0	0.0	3.6	6.9	2.6	2.6
	100	0.0	0.0	0.0	0.0	18.5	36.0	13.5	13.5
	200	2.6	18.5	2.6	5.0	36.0	50.0	36.0	70.0
Cucumber	25	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.8
	100	1.6	4.1	2.6	11.0	32.5	35.0	1.5	4.8
	200	8.5	2.6	8.5	10.5	17.0	4.4	5.3	11.0
Pepper	50	0.0	0.0	3.2	4.3	3.2	3.5	0.0	0.0
	100	0.0	0.0	3.7	2.1	2.2	7.8	3.8	6.2
	200	2.7	4.5	5.0	7.4	5.5	12.5	4.2	6.2
	500	2.9	4.6	7.8	6.9	11.5	20.5	19.0	35.0

plants: tomato, cucumber, and pepper. When the seeds were simply dipped instead of soaked, the concentration needed for the antibiotic to be detected was 200 μg/ml for tomato and pepper seedlings, and 100 μg/ml for cucumbers. As the period of immersion was increased, lower concentrations furnished sufficient antibiotic for movement into the roots and young leaves, but in no case were concentrations below 25 μg/ml effective. A 12-hour treatment at 100 μg/ml, for example, allowed accumulation of the following concentrations of antibiotic in the roots and leaf, respectively: tomato, 36 and 19 μg/ml; cucumber, 35 and 32 μg/ml; and pepper, 13 and 5 μg/ml. Higher concentrations resulted in still greater accumulations in the plant parts.

To study the duration of the antibiotic translocated fror., seed to root and crown, and to determine whether it would travel to higher levels, seeds were treated with solutions of 500 and 1000 μg/ml and planted in sterile soil. In cucumber, peas, and Lima bean treated with 1000 μg/ml, the antibiotic could be detected in root and shoot for as long as 20 days after planting, and in leaves of Lima bean for 30 days. Even after this extended period, the concentrations of vancomycin were in a range sufficient to inhibit many of the pathogens. In leaf and root, the respective concentrations in plant juices at 20 days were: cucumber, 2.4 and 1.6 μg/ml; pea, 2.1 and 1.8 μg/ml; Lima bean, 9.0 and 6.3 μg/ml. At 30 days the concentration in the bean leaves was 4.1 μg/ml. At 10 days the antibiotic was found in all parts of the tomato plant, but at 20 days had disappeared from the root. The concentration of 500 μg/ml was not as effective as 1000 μg/ml; concentrations in leaves, stem, and root of peas and Lima bean were detectable at 5 days after planting, but not later, except for leaves of Lima bean, where some antibiotic persisted for 15 days.

Absorption from soil.—The rapid absorption of vancomycin by roots suggested the possibility of supplying the antibiotic to plants through soil water. Cucumber and pea plants in black sandy loam were watered daily with 100 ml of vancomycin in one of two concentrations: 200 or 500 μg/ml. Water was used as a control. The leaves, stems and roots were assayed after 0, 6, 12, 24, 48, 72, and 96 hours. After 2 daily applications to peas the following concentrations were contained in the roots: 6.7 and 4.5 μg/ml for the 200- and 500-μg treatments, respectively. The content after 3 daily soil treatments, in the same order, was 8.3 and 70 μg/ml, and 4 treatments resulted in the accumulation of 17 and 46 μg/ml. Concentrations in the crown of the plants for the 200- and 500-μg treatments, respectively, were: 1.7 and 2.7 for 2 daily applications, 2.1 and 4.0 for 3 applications, and 4.7 and 5.0 μg/ml plant juice for 4 applications. Vancomycin was contained in cucumber roots only after 3 days at the 500-μg treatment—6.7 μg/ml. A fourth application resulted in the accumulation of 4.7 and 5.0 μg/ml from the 200- and 500-μg applications, respectively. The antibiotic was not present in the crown even after 4 treatments.

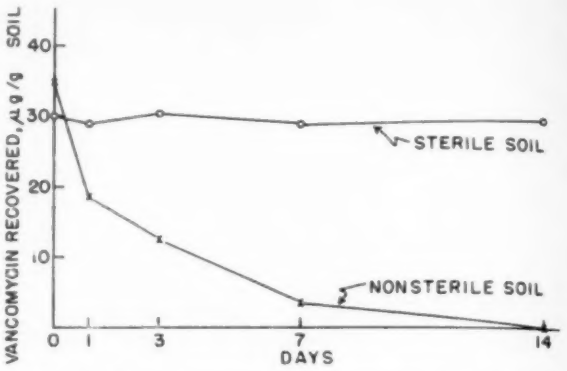


Fig. 2. The disappearance of vancomycin from sterile and unsterile soils.

Soil inactivation.—Preliminary studies indicated that at least 2 mechanisms for inactivation in the prairie loam soil might account for the comparatively small amount of absorption of vancomycin by plants grown in this environment. To determine the role of the microflora in the disappearance of vancomycin, the procedure of Gottlieb and Siminoff (7) was followed with certain variations. After the antibiotic was added to both sterile and unsterile soil, samples were taken at various intervals. Although 50 μg of antibiotic were added per g of air-dried soil, only about 30 μg could be recovered at zero time. In sterile soil the vancomycin content remained constant for 14 days, but in unsterile soil gradually decreased until, at 14 days, it was not detectable (Fig. 2). The adsorptive properties of the soil were studied by adding 5 ml vancomycin at concentrations of 25 and 125 μg/ml per g of soil. The suspensions were then agitated on a rotary shaker for periods of 0–24 hours, and the supernatant fluids assayed. At 0.5, 3.0, and 24 hours, the respective percentages of antibiotic still active in the aqueous solution were 24, 10, and 7.5. Obviously, the antibiotic could disappear from the soil both by the action of soil microflora and by adsorption. Further studies will be necessary to explain the disparity between the large amounts lost with the soil-suspension technique and the small amounts in the studies on the role of microflora.

Discussion.—A number of properties of vancomycin converge to place importance on the possible role of this antibiotic in the prevention and control of plant diseases. That it inhibits not only gram-positive but many gram-negative bacteria widens its potential value. The sensitivity of the *Corynebacteria* was not surprising, since the antibiotic inhibits many other gram-positive bacteria. However, its ability to suppress the growth of many gram-negative plant-pathogenic bacteria indicates the need for further study on the effectiveness of vancomycin in the control of diseases produced by such bacteria. Data presently available indicate that only short periods of exposure are necessary to kill sensitive organisms (18), in contrast to the period needed when microorganisms

are exposed to many other antibiotics, such as penicillin and streptomycin, which are primarily bacteriostatic. Furthermore, there has been no indication that organisms acquire resistance to vancomycin, despite attempts to develop resistant strains (18). Such an antibiotic could then be effective even if low concentrations were present in a plant tissue for relatively short periods. Nevertheless, the potential of any antibacterial or antifungal agent to control disease cannot be accurately evaluated by its ability to inhibit the growth of microorganisms *in vitro* (14).

In common with some other antibiotics, vancomycin can be absorbed by the leaves and stems of plants. Vancomycin is not unique in being translocated upward in the plant from the point of application. The relationship between concentrations of the antibiotic supplied to the plant and the amount absorbed tends to be linear, similar to the behavior of chloramphenicol and streptomycin in broad beans (15), and griseofulvin in both the broad bean and tomato (5). The inability of the plant to absorb greater concentrations of vancomycin when glycerol is present is a property in common with oxytetracycline and neomycin. This is in contrast to streptomycin, chloramphenicol, and streptothricin, whose intake is enhanced in the presence of glycerol (9). The ineffectiveness of this humectant in increasing absorption is puzzling because this additive is supposed to act by increasing the water available on the plant surface, thus keeping the antibiotic in solution for longer periods. Since vancomycin is a water-soluble agent, one would have expected glycerol to favor its intake. The lack of increased absorption with glycerol also makes difficult an explanation for the greater absorption of the antibiotic at 10:00 P.M. than when sprayed on plants at other times. One would be tempted, otherwise, to explain the phenomenon by the slow evaporation of water from the vancomycin solution during the night.

A unique property of vancomycin is the ease with which it is translocated downward in the plant from the area where applied. Only a few antibiotics, such as streptothricin and pleocidin, move in this manner (10). This downward movement is very marked with vancomycin, for it not only accumulates in lower leaves but also readily reaches the roots. Even when only 1 leaf is sprayed, the concentrations in the root are often greater than needed to inhibit a number of plant-pathogenic bacteria *in vitro*. This preferential translocation is also observed in the movement of vancomycin out of treated seeds, allowing a greater accumulation of the active substance in the roots than the shoots of the young plant. Another significant feature is the ease with which the inhibitory material is absorbed by the root and moved to the upper plant parts. Absorption by roots is so rapid that the concentrations become greater in these tissues than in the nutrient solution. Furthermore, this characteristic makes possible the absorption of vancomycin from soil even though the antibiotic can be both inactivated by adsorption and degraded by soil microflora.

The ability of plants to withstand high concentrations of vancomycin is outstanding. In no instance was toxicity observed in any of the 7 types of plants used in the experiment. Even a concentration of 1000 μg/ml of root juice was not toxic. No chlorosis, stunting of growth, depression of germination, or other abnormal characteristics were observed.

DEPARTMENT OF PLANT PATHOLOGY
AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

LITERATURE CITED

1. AMMANN, A., AND D. GOTTLIEB. 1955. Paper chromatography of antifungal antibiotics. Appl. Microbiol. **3:** 181-186.
2. ANDERSON, H. W., AND INEZ NEINOW. 1947. Effect of a streptomycin on higher plants. Phytopathology **37:** 1.
3. BRIAN, P. W., J. M. WRIGHT, J. STUBBS, AND A. M. WAY. 1951. Uptake of antibiotic metabolites of soil microorganisms by plants. Nature **167:** 347.
4. CROWDY, S. H., AND D. PRAMER. 1955. Movement of antibiotics in higher plants. Chem. & Ind. (London) (Rev.). **7:** 160-162.
5. CROWDY, S. H., J. F. GROVE, H. G. HEMMING, AND KATHLEEN C. ROBINSON. 1956. The translocation of antibiotics in higher plants. II. The movement of griseofulvin in broad bean and tomato. Exptl. Botany. **7:** 42-64.
6. DUNEGAN, J. C., AND R. A. WILSON. 1956. Preliminary note on the downward movement of streptomycin in apple and pear tissue. Plant Disease Reptr. **40:** 478.
7. GOTTLIEB, D., AND P. SIMINOFF. 1952. The production and role of antibiotics in the soil. II. Chloromycetin. Phytopathology **42:** 91-97.
8. GRAY, R. A. 1955. The downward translocation of antibiotics in plants. Supplement to the proceedings of the 6th plant physiology meeting. (Abstr.) Plant Physiol. **30.**
9. GRAY, R. A. 1956. Increasing the absorption of streptomycin by leaves and flowers with glycerol. Phytopathology **46:** 105-11.
10. GRAY, R. A. 1958. Downward translocation of antibiotics in plants. Phytopathology **48:** 71-78.
11. HAMILTON, J. M., M. SZKOLNICK, AND E. SODENHEIMER. 1956. Systemic control of cherry leaf spot fungus by foliar sprays of Acti-dione derivatives. Science **123:** 1175.
12. LOO, Y. H., P. S. SKELL, H. H. THORNBERRY, J. ERLICH, J. M. MCGUIRE, G. M. SAVAGE, AND J. C. SYLVESTER. 1945. Assay of streptomycin by the paper disc plate method. Bacteriol. **50:** 701-709.
13. MCCORMICK, M. H., W. M. STARK, G. E. PITTENGER, R. C. PITTENGER, AND J. M. MCGUIRE. 1955-56. Vancomycin, a new antibiotic. I. Chemical and biological properties. Antibiotics Ann. **1955/56:** 606-611.
14. NATTI, J. J. 1957. Control of downy mildew of broccoli with antibiotics and fungicides. Plant Disease Reptr. **41:** 780-788.
15. PRAMER, D. 1954. The movement of chloramphenicol and streptomycin in broad bean and tomato plants. Am. J. Botany **18:** 463-470.
16. PRAMER, D. 1954. Observations on the uptake and translocation of five actinomycete antibiotics by cucumber seedlings. Ann. Appl. Biol. **40:** 617-622.
17. SMITH, D. S., C. B. LANDERS, AND J. FORGACS. 1950. A cylinder plate assay for chloromycetin in body fluids and tissue extracts. J. Lab. Clin. Med. **36:** 154-157.
18. ZIEGLER, D. W., R. W. WOLFE, AND J. M. MCGUIRE. 1955-1956. Vancomycin, a new antibiotic. II. In vitro antibacterial studies. Antibiotics Ann. **1955/-56:** 612-618.

WHITE TIP DISEASE OF RICE. II. SEED TREATMENT STUDIES[1]

E. H. Todd[2] and John G. Atkins[3]

SUMMARY

Infested seed serve as the source of inoculum for the white tip disease of rice, caused by *Aphelenchoides besseyi* Christie, 1942. Hot water and fumigation with methyl bromide, and a large number of chemicals were evaluated as treatments for eliminating the seed-borne nematodes. Fifteen minutes at 55–61°C was effective as 15 minutes at 53°C after presoaking, and caused less seed injury. Methyl bromide, tested as a fumigant at several rates and exposure periods on rice seed of various moisture levels, was not satisfactory. Among a large number of chemicals evaluated in laboratory tests, experimental chemical N-244 (10% 3-*p*-chlorophenyl-5-methyl rhodanine) was most promising in nematocidal action and lack of phytotoxicity. Later samples, from batches made on a production basis, were ineffective for control of white tip.

White tip, caused by *Aphelenchoides besseyi* Christie, 1942 (syn. *A. oryzae* Yokoo, 1948), has been an important rice disease in the southern rice states in susceptible varieties. The nematodes are seed-borne. Since, as far as known, infested seed serve as the only source of inoculum (7), this disease theoretically might be controlled by eliminating the source of inoculum. Seed treatment studies for control of white tip were initiated in 1949. A preliminary report has been published (1).

In 1948 Yokoo (10) described *A. oryzae* Yokoo as a seed-borne nematode of rice in Japan. In 1949 Cralley (2) reported that the white tip disease in the United States was caused by a seed-borne nematode similar to *A. oryzae*. He obtained good control of white tip in greenhouse tests by a hot-water seed treatment of 52–53°C for 15 minutes. In 1951 Yoshii and Yamamoto (11) found 2 hot-water treatments effective: 1) immersion for 5–10 minutes at 50–52°C after the seed had been soaked 16–20 hours in water below 20°C; and 2) direct immersion of dry seed for 10–15 minutes in water at 56–57°C. In 1952 Cralley (3) reported yield increases of 5–10 bu./A. in the susceptible Arkrose variety from hot-water treatment (15 minutes at 50–53°C after 8–12 hours in cool water).

Tullis (8) considered fumigation with methyl bromide to be an effective control measure when used at 1.25 lb. per 1000 cu. ft. for 12 hours. He (9) later suggested either a dose of 1.25 lb. per 1000 cu. ft. for 15 hours or a double treatment: 1 lb. per 1000 cu. ft. for 15 hours, aeration for several days, and then 0.5 lb. per 1000 cu. ft. for 15 hours. Cralley (3), in 1952, concluded that the methyl bromide dose required to kill nematodes varied inversely with the moisture content of the rice seed. Rice seed containing 14% moisture was more likely to be damaged by methyl bromide than seed lots of a lower moisture level.

Cralley and French (4) significantly decreased white tip severity by using certain chemicals as seed treatments, as well as by methyl bromide fumigation. These were parathion dust (*0,0*-diethyl-*0*-*p*-nitrophenyl phosphorothioate, 25%), Systox dust (*0,0*-diethyl-*0*-2-ethylmercapto diethyl thiophosphonothioate, 50%), and Aagrano dust ((3-ethoxypropyl) mercury bromide, 3.5%) at 2 oz./bu. and a 12-hour soak in 1:1000 mercury bichloride. This last dust and soak gave some mercurial toxicity on rice seedlings.

MATERIALS AND METHODS.—In evaluating the treatments as to nematocidal action, samples of treated seed were examined for viable nematodes. The hulls and kernels from 10-grain samples were placed in Syracuse watch glasses or 50-mm Petri dishes in 10–15 ml of water. After 4–8 hours, the containers were examined under a stereoscopic microscope for motile nematodes. Those showing no motion were classed as dead, although such a classification was sometimes doubtful. The various seed lots varied as to nematode infestation.

The effects of the treatments on seed viability were determined by a laboratory germination test. Replicated 50-grain samples were placed on moist filter paper in Petri dishes and held at 30°C for rapid germination. A decrease in normal germination was used as a criterion for phytotoxicity.

EXPERIMENTAL RESULTS.—Various seed treatments were evaluated in the laboratory as control measures for the seed-borne nematode. Basically, the various treatments were evaluated as to 1) killing of the nematodes and 2) effect on seed germination. These tests are grouped as 1) hot water, 2) methyl bromide fumigation, and 3) screening of chemical compounds.

Hot-water seed-treatment tests.—Small lots of infested rice seed, generally 30-g samples in small muslin bags, were treated in a thermostatically controlled

[1] Accepted for publication October 28, 1958.

Based in part on a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree from Louisiana State University, 1953.

The writers acknowledge the assistance of N. E. Jodon and R. K. Walker, Rice Experiment Station, Crowley, Louisiana; the Dow Chemical Company; and the Stauffer Chemical Company. The 1953 and 1954 tests were in cooperation with the Rice-Pasture Experiment Station and the Texas Rice Improvement Association, Beaumont, Texas.

[2] Formerly graduate student and research associate at Louisiana State University; presently pathologist, Crops Research Division, ARS, U. S. D. A., Canal Point, Florida.

[3] Formerly assistant plant pathologist, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana; presently pathologist, Crops Research Division, ARS, U. S. D. A., Beaumont, Texas.

water bath equipped with a thermometer and stirring attachment. The samples were then examined for viable nematodes and checked for germination.

A study using presoaked seed (24 hours in cool water) treated at 49–55°C for 5, 10, and 15 minutes showed that 51 and 53°C were effective without seriously reducing germination. Treatment at 49°C for 5 and 10 minutes failed to kill all of the nematodes. Germination was reduced at the highest temperature, 55°C, for 15 minutes. It was concluded that 15 minutes at 51–53°C could be used for treating large batches of rice seed if equipment was not available for more precise temperature control.

In another study the presoak treatment was omitted, and infested seed was treated in hot water at 55, 57, 59, and 61°C for periods of 5, 10, and 15 minutes. The temperatures necessary for killing all nematodes were higher than those necessary when the rice was presoaked. However, injury to the seed was less. Temperatures of 55–61°C for 10 and 15 minutes killed all nematodes without reduction in germination. At the 5-minute exposure period all temperatures except 55°C were effective in killing nematodes. The results obtained indicated that the presoak period could be eliminated and that less seed injury would be encountered at effective temperatures.

Methyl bromide fumigation tests.—Since methyl bromide is generally considered an effective nematocide and is widely used in fumigating grain for insects, the material was evaluated for killing the nematode that causes white tip.

A special laboratory fumigation system was constructed as designed by the Dow Chemical Company, Midland, Michigan. As fumigation vaults, 1-liter Erlenmeyer flasks were used for 30-g samples of infested rice treated in quadruplicate. Two lots of seed were used at moisture levels of 11.8% and 14.9%. Samples were treated for 12 and 18 hours at 1.0, 1.5, and 2.0 lb./1000 ft.^{3} Following fumigation, the samples were aerated 24 hours before evaluation as to nematode kill and effect on germination. None of the treatments gave complete or nearly complete kill of nematodes without drastically reducing or retarding germination.

In further tests, the effect of moisture level of seed on efficiency of methyl bromide fumigation was studied in cooperation with the Dow Chemical Company, Midland, Michigan, and E. M. Cralley, Plant Pathology Department, University of Arkansas. The rice was treated by the Dow Chemical Company. Duplicate samples were sent to the writers and to Dr. Cralley. The test involved seed of 3 moisture levels, 3 fumigation periods, and 5 different rates of methyl bromide.

The treated samples were examined in the laboratory so as to determine the effect on nematodes and seed germination (Table 1). In seed at a moisture level of 9.7%, nematode kill was not satisfactory at any rate. At the highest moisture level, 17.7%, germination was reduced at all rates. At a seed moisture content of 13.8% some of the treatments gave nearly complete kill of the nematodes without serious injury to the seed. It is probable that certain rates, for example, 1.5 lb. for 12 hours, would reduce white tip without drastic reduction in emergence if fumigation is precise.

TABLE 1.—*Viability of nematodes and germination of rice seed of 3 moisture levels after fumigation with methyl bromide*

Seed moisture content	Treatment period	Dose (lb./100 ft.^{3})	Viable nematodes[a] (no.)	Seed germination[a] (%)
9.7%	6 hours	1.00	21	92
		1.25	22	94
		1.50	18	94
		1.75	22	92
		2.00	22	91
	12 hours	1.00	20	92
		1.25	14	90
		1.50	24	91
		1.75	22	90
		2.00	22	89
	24 hours	1.00	6	88
		1.25	19	92
		1.50	10	85
		1.75	24	89
		2.00	23	80
		Control	40	94
13.8%	6 hours	1.00	8[b]	89
		1.25	5[b]	91
		1.50	10[b]	84
		1.75	4[c]	87
		2.00	8[c]	86
	12 hours	1.00	7[c]	86
		1.25	0	79
		1.50	6[c]	72
		1.75	4[c]	64
		2.00	0	49
	24 hours	1.00	0	52
		1.25	0	16
		1.50	0	11
		1.75	0	11
		2.00	0	31
		Control	23	95
17.7%	6 hours	1.00	9[b]	64
		1.25	13[c]	53
		1.50	9[c]	32
		1.75	4[c]	38
		2.00	7[c]	23
	12 hours	1.00	0	15
		1.25	0	0
		1.50	0	0
		1.75	0	0
		2.00	0	0
	24 hours	1.00	0	0
		1.25	0	0
		1.50	0	0
		1.75	0	0
		2.00	0	0
		Control	18	96

[a] Each value an average for 4 samples.
[b] Nematodes sluggish.
[c] Nematodes very sluggish.

It was concluded that methyl bromide is not a practical control for white tip, since the kill of nematodes would probably be erratic and the treatment would be hazardous to germination.

Laboratory tests of chemicals.—A fairly large number of chemicals were evaluated in a laboratory screening program as possible seed-treatment nematocides. These were primarily fungicides, insecticides,

or nematocides on the market as commercial products, or experimental chemicals available for testing in 1949 and 1950.

In the tests, small samples of infested seed were treated, held several days in closed containers, and evaluated for effectiveness of treatments. In the preliminary tests, chemicals formulated for dust or slurry application were used at the maximum holding capacity of the seed. Liquid materials were tested at what was considered excessive rates. These laboratory tests using excessive rates were conducted to select chemicals exhibiting good nematocidal action. Those giving complete or nearly complete nematode kill were designated promising and retested in other screening tests. Their effect on seed germination also was determined.

The names and ingredients of the 55 chemical compounds used in the screening tests are not listed. Non-mercurial fungicides, such as thiram (bis(dimethylthiocarbamoyl) disulfide, 50%), chloranil (tetrachloro-*p*-benzoquinone, 48%), and dichlone (2,3-dichloro-1,4-naphthoquinone, 50%), showed little or no nematocidal action. Certain mercurial fungicides, such as Panogen (cyano(methylmercuri)guanidine, 2.1%), Ceresan M (*N*(ethylmercuri)-*p*-toluenesulfonanilide, 7.7%), New Improved Ceresan (ethylmercury phosphate, 5%), and Aagrano, killed the nematodes but drastically reduced germination at the rates used. Chloropicrin, ethylene dibromide, and formalin killed all nematodes, but seed germination was zero. A group of insecticides, including parathion, killed some of the nematodes, but these materials were not tested further. Three experimental rhodanine compounds, N-168 (3-methyl-5-ethyl rhodanine, 10%), N-244 (3-*p*-chlorophenyl-5-methyl rhodanine, 10%), and N-245 (3-*p*-chlorophenyl-5-ethyl rhodanine, 10%), were rated as most promising for nematode kill and lack of phytotoxicity.

Some of the more promising materials were included in further tests at known rates. The results obtained in one representative test series are given in Table 2. N-244 and N-245 again appeared to be promising for white tip control. These materials were then included in more extensive laboratory tests. As shown in Table 3, N-244 and N-245 at 4 oz./bu. killed all nematodes without reducing or inhibiting seed germination. N-168 was less effective.

N-168, N-244, and N-245 were the most promising of the various chemical compounds tested in the laboratory as seed treatments for the control of white tip. They were then selected for field testing along with certain other treatments.

Seed treatment field tests.—A triplicated field test of 3 treatments was conducted at the Rice Experiment Station, Crowley, Louisiana, in 1952. The treatments used and the results obtained are given in Table 4. Severe white tip did not develop in any of the plots, and only 1 of the 3 replications of the hot-water and N-244 treatments showed any white tip. In this repli-

TABLE 2.—*Viability of nematodes and germination of rice seed treated with chemicals at 2 dose rates*

Chemical and dose	Active ingredient	Relative activity of nematodes[a] (class)	Seed germination[b] (%)
Ceresan M	(ethylmercuri)-*p*-toluenesulfonanilide, 7.7%		
Maximum capacity		0	0
1 oz./bu.		1	83[c]
New Improved Ceresan	ethylmercury phosphate, 5%		
Maximum capacity		0	0
1 oz./bu.		1	85[c]
Experimental Chemical F-800	unknown		
Maximum capacity		0	0
1 oz./bu.		1	67[c]
Dow 9B	zinc 2,4,5-trichlorophenoxide, 50%		
Maximum capacity		0	0
1 oz./bu.		1	70[c]
Panogen	cyano(methylmercuri)guanidine, 2.1%		
Excessive rate		0	0
2 oz./bu.		1	74[c]
Aagrano	(3-ethoxypropyl)mercury bromide, 3.5%		
Maximum capacity		0	0
2 oz./bu.		1	50[c]
Experimental Chemical N-168	3-methyl-5-ethyl rhodanine, 10%		
Maximum capacity		1	85
2 oz./bu.		2	89
Experimental Chemical N-244	3-*p*-chlorophenyl-5-methyl rhodanine, 10%		
Maximum capacity		0	86
2 oz./bu.		1	88
Experimental Chemical N-245	3-*p*-chlorophenyl-5-ethyl rhodanine, 10%		
Maximum capacity		0	84
2 oz./bu.		1	91
Control		3	87

[a] Relative activity, classes 0–3.
[b] Each value an average for 2 samples.
[c] Retarded germination.

TABLE 3.—*Viability of nematodes and germination of rice seed treated with 3 experimental rhodanine chemicals containing 10% of the active ingredient*

Chemical and dose	Viable nematodes[a] (no.)	Relative activity of nematodes[b] (class)	Seed germination[a] (%)
N-168			
2 oz./bu.	11	3	90
3 oz./bu.	4	2	85
4 oz./bu.	7	2	88
N-244			
2 oz./bu.	2	1	89
3 oz./bu.	1	1	91
4 oz./bu.	0	0	87
N-245			
2 oz./bu.	6	3	93
3 oz./bu.	5	2	89
4 oz./bu.	0	0	86
None (control)	8	4	89

[a] Each value an average for 4 samples.
[b] Relative motility of nematodes, classes 0–4.

cation, the amount of infection, based on foliar symptoms, was markedly reduced. Some or all of the infection in treated plots may have resulted from breaks in levees from a heavy rainfall.

In 1953 a field test was conducted at the Rice-Pasture Experiment Station, Beaumont, Texas, with seed sown with a drill or in water.[4] Following treatment, the seed was held 72 hours in sealed containers before drill-seeding or soaking in water in preparation for water-seeding. All plots were separated by an earth or a metal levee. The results (Table 5) again demonstrated the effectiveness of N-244 (10%) at 4 oz./bu. and hot water (60°C for 15 minutes without presoak treatment). Dow 6907, which had shown promise in some supplemental laboratory tests, reduced white tip but markedly retarded germination and initial vigor.

On the basis of laboratory tests and the 1952 and 1953 tests with Experimental Chemical N-244 (active ingredient, 10%), the material was made available for use by the rice farmers (5, 6). The commercial product, Stauffer Rice Seed Treatment #1, contained 40% of the active ingredient.

In 1954 the ineffectiveness of commercial batches of N-244 (40%) was demonstrated in Arkansas[5] by E. M. Cralley. A limited field test was conducted in Beaumont, Texas, in 1955 with a slurry N-244 (40%) at 1.25 oz./bu. as a treatment on infested Arkrose rice. White tip was not controlled effectively, nor was this material effective[5] in the 1955 test at the Rice Branch Experiment Station, Stuttgart, Arkansas.

DISCUSSION.—The hot-water treatment appears to be the most reliable for small seed lots, but it is not practical for large-scale treatment of commercial seed rice. The hot-water treatment has been used as needed for "cleaning up" lots of infested seed rice at the 3 southern rice experiment stations—at Beaumont, Texas; Crowley, Louisiana; and Stuttgart, Arkansas.

[4] Soaked rice seeded broadcast in muddy water; water drained within 48 hours.
[5] Unpublished data; based on oral report of E. M. Cralley and inspection of plots by junior author.

TABLE 4.—*Effect of 3 treatments on viability of nematodes, seed germination, and incidence of white tip in field plots*

Treatment	Viable nematodes[a] (class)	Seed germination[b] (%)	White tip incidence[c] (class)
Hot water, 53–60°C, 14 minutes	0	91	0.3
Aagrano, 2 oz./bu.	2	44	1.0
N-244 (10%), 4 oz./bu.	0	88	0.3
Control	3	90	2.0

[a] Each value an average for 2 samples, classes 0–3.
[b] Each value an average for 4 samples.
[c] Each value an average for 3 replications, classes 0–5.

In 1949 when the studies were initiated, the writers envisaged a treatment sufficiently simple and inexpensive for annual treatment of seed rice for several thousand acres. However, later experience with the white tip disease has shown that annual treatments are not necessary. Disease-free seed produced on foundation and certified seed fields is presently a means of preventing serious losses in commercial fields.

In evaluating the various seed treatments, the writers used the hot-water treatment as a standard for comparison. Thus, to be considered promising, a treatment had to give essentially 100% nematode kill with little or no seed injury. Consequently, several materials that might give partial control of white tip were probably discarded as ineffective. It seems probable that the methyl bromide treatments suggested by Tullis (8, 9) would reduce white tip somewhat. Methyl bromide was discarded because none of the treatments compared favorably with the hot-water method, and the tests indicated the dose-time range to be rather narrow. Also, as Cralley (3) reported, the effective methyl bromide dose varies inversely with the moisture content of the rice seed. Certain mercury fungicides, such as Ceresan M, Panogen, and Aagrano, are nematocidal, but only at phyto-

TABLE 5.—*Incidence of white tip in rice grown from treated seed using 2 seeding methods*

Type of seeding and treatment	Treatment dose	White tip incidence[a] (class)
Drill seeding		
N-244	4.0 oz./bu.	0.2
Dow 6907[b]	0.5 oz./bu.	0.7
Control		5.0
Water seeding		
N-244	4.0 oz./bu.	0.2
N-244 + sticker	4.0 oz./bu.	0.0
N-244	2.0 oz./bu.	2.0
N-244 + sticker	2.0 oz./bu.	1.2
Dow 6907[b]	1.0 oz./bu.	0.2
Dow 6907[b]	0.5 oz./bu.	0.5
Hot water	60°C, 15 minutes	0.0
Control		4.5

[a] Each value an average, classes 0–5.
[b] Experimental chemical, Dow Chemical Company.

toxic rates. Lower rates were not considered to be effective or promising enough for field testing. Parathion and similar insecticides were not investigated fully as practical seed treatments, because of possible health hazards to people engaged in treating and seeding operations.

After N-244 was discovered to be a promising chemical in the laboratory screening tests, further studies were largely confined to its evaluation in laboratory and field tests. The original experimental material appeared to be the ideal chemical in that it 1) approached the hot-water treatment in kill of nematodes, 2) caused no seed injury over a reasonable dose range, 3) could be used as a simple dust or a slurry treatment, and 4) did not create a health hazard. Since experimental samples of N-244 (10% active ingredient) gave control of white tip in Louisiana as well as in Arkansas (3), the material was recommended for use. In the writers' opinion, the data obtained on the effectiveness of experimental samples of N-244 were reliable. The reasons for subsequent poor performance of commercial batches of N-244 (Stauffer Rice Seed Treatment No. 1) are not known.

Department of Plant Pathology
Louisiana Agricultural Experiment Station
Baton Rouge, Louisiana
and
Crops Research Division
Agricultural Research Service
U. S. Department of Agriculture

LITERATURE CITED

1. Atkins, J. G., and E. H. Todd. 1952. Laboratory screening of chemicals for control of rice white tip. (Abstr.) Phytopathology **42**: 463.
2. Cralley, E. M. 1949. White tip of rice. (Abstr.) Phytopathology **39**: 5.
3. Cralley, E. M. 1952. Control of white tip of rice. Arkansas Farm Research I (1): 6.
4. Cralley, E. M., and R. G. French. 1952. Studies on the control of white tip of rice. (Abstr.) Phytopathology **42**: 6.
5. Smith, H. E., and E. M. Cralley. 1953. Rice diseases. Arkansas Agr. Ext. Ser. Leaflet 198, p. 1-4.
6. Stroube, W. H. 1954. Control of white tip of rice by seed treatment. Louisiana Agr. Expt. Sta. Annual Report 1952-1953, p. 114-115.
7. Todd, E. H., and J. G. Atkins. 1958. White tip disease of rice. I. Symptoms, laboratory culture of nematodes, and pathogenicity tests. Phytopathology **48**: 632-640.
8. Tullis, E. C. 1951. Diseases of rice. U. S. Dept. Agr. Farmers' Bull. No. 1854, p. 1-16.
9. Tullis, E. C. 1951. Control of the seed-borne nematode of rice by fumigation with methyl bromide. Texas Agr. Expt. Sta. Progr. Rept. 1413, p. 1-4.
10. Yokoo, T. 1948. Aphelenchoides oryzae Yokoo n. sp., a nematode parasitic to rice plant. Ann. Phytopathol. Soc. Japan **13**: 40-43.
11. Yoshii, H., and S. Yamamoto. 1951. On some methods for the control of the rice nematode disease. Sci. Bull. Fac. Agr. Kyushu Univ. **12** (2): 123-131.

WHITE TIP DISEASE OF RICE. III. YIELD TESTS AND VARIETAL RESISTANCE[1]

John G. Atkins[2] and E. H. Todd[2, 3]

SUMMARY

White tip, caused by *Aphelenchoides besseyi* Christie, 1942, resulted in yield reductions up to 40–50% in such susceptible rice varieties as Blue Rose and Early Prolific in field plots artificially infested with laboratory cultures of nematodes. Over a 3-year period, yields were reduced 17% in susceptible varieties and 7% in resistant varieties. In other tests, 28 rice varieties were classified as to white tip reaction under both natural and inoculated test conditions. Seed samples from resistant varieties grown in isolation plots, from nematode-infested seed, rarely contained nematodes.

White tip, caused by *Aphelenchoides besseyi* Christie, 1942, has for many years been an important rice disease in Arkansas, Louisiana, and Texas. The disease probably caused its greatest losses and the most concern to the rice growers during the period 1935–1945, when Early Prolific and Blue Rose were important varieties. In the last 10 years, white tip has been less important, because of resistant and less susceptible varieties, together with control measures.

Jodon (6), in 1935, was apparently the first to recognize white tip as a disease to be considered in the rice breeding program. Since then, Jodon and various other rice research workers have observed and reported differences in reaction to white tip among varieties and selections. The severity of symptoms varied greatly from year to year under conditions of natural infection, and ratings were frequently inconsistent. As a result, a variety might be rated as susceptible at one location and fairly resistant at another. Since white tip was regarded as a physiological disease until 1949, such inconsistent information was accepted as due to differences in environment or soil conditions.

Concepts regarding varietal reaction tests changed after 1949, when white tip was classed as a seed-borne nematode disease. In recent years, heavily infested seed of a susceptible variety for "spreader" rows and laboratory cultures of nematodes have been used in special nurseries consisting of varieties and selections to be tested for resistance.

Prior publications by the writers (9, 10) and by Cralley (4) described disease symptoms, established the disease as being caused by a seed-borne nematode, *A. besseyi*, and reported successful control by seed treatment. Cralley (5) obtained yield increases of 5–10 bu./A. from the hot-water seed treatment in field plots using Arkrose, a susceptible variety. Several papers (1, 2, 3, 7, 8) list many of the rice varieties as resistant or susceptible to white tip.

EFFECT OF WHITE TIP ON YIELDS.—Although white tip has been considered an important rice disease in the United States, only Cralley (5), using a single variety, has reported on its effect on yield. Tests were conducted at the Rice-Pasture Experiment Station, Beaumont, Texas, in 1954, 1955, and 1956 to determine the effect of white tip on the yields of the important rice varieties. The varieties tested included not only those generally considered susceptible but also those rated as resistant because they rarely showed foliar or panicle symptoms typical of white tip. The seed of some varieties considered resistant contained nematodes, and their yields might be reduced by the nematodes.

Each of the 15 test varieties was seeded as 4-row subplots 17 ft. long in each of the 6 $^1/_{20}$-A. plots. A levee surrounded each plot, and individual irrigation and draining were provided to prevent movement of nematodes from plot to plot in irrigation water. Before seeding, the seed was treated to eliminate seed-borne nematodes with N-244[4] in 1954 and 1955, and with hot water[5] in 1956. About 5 weeks after seeding, 3 of the plots were heavily infested with laboratory cultures of nematodes, and all 6 plots irrigated to a shallow depth. Several pounds of inoculum (nematodes plus substrate) were used in the test plots. Several weeks earlier, laboratory cultures of *A. besseyi* had been established in 1- or 2-liter flasks. The techniques used in obtaining laboratory cultures of this nematode are given in a previous publication (9).

Before heading, all plots were visually rated as to severity of white tip. In the 1954 test, the disease was heavy on susceptible varieties in the three white-tip-inoculated plots. The same varieties showed only a trace of white tip in the control plots. The resistant varieties showed limited atypical leaf symptoms.

Table 1 gives varieties, average yields, and disease ratings for the 1954 test. As shown, white tip caused yield losses of 17–54% in the susceptible varieties and 0–24% in the resistant varieties.

In the 1955 and 1956 tests, white tip was only moderately severe on the susceptible varieties. Average yield reductions in the two groups of varieties,

[1] Accepted for publication October 29, 1958.

Cooperative investigations of the U. S. D. A., ARS, Crops Research Division; the Agricultural Experiment Stations of Arkansas, Louisiana, and Texas; and the Texas Rice Improvement Association.

The writers acknowledge the assistance of H. M. Beachell, Research Agronomist, Crops Research Division, ARS, U. S. D. A., in providing seed of the rice varieties and suggestions for the field tests.

[2] Pathologists, Crops Research Division, ARS, U. S. D. A.

[3] Present address U. S. D. A. Sugarcane Field Station, Canal Point, Florida.

[4] 3-*p*-chlorophenyl-5-methyl rhodanine, 10%, at 4 oz./bu.

[5] Fifteen minutes at 58–60°C.

TABLE 1.—*Effect of white tip on yield of resistant and susceptible rice varieties*

Variety	C.I. no.[a]	Variety reaction[b]	Yield (lb./A.)		Reduction	
			White tip	Control	(lb.)	(%)
Early Prolific	5883	S	1810	3941	2131	54.1
Blue Rose	2128	S	1900	3406	1506	44.2
Arkrose	8310	S	3194	4563	1369	30.0
Calrose	8988	S	2029	3681	1652	44.9
Caloro	1561-1	S	3084	4078	994	24.4
Magnolia	8318	S	2389	2891	502	17.4
Zenith	7787	S	1991	3504	1513	43.2
Century Patna 231	8993	MR	2378	3095	717	23.2
Bluebonnet 50	8990	R	3575	4282	707	16.5
Improved Bluebonnet	8992	R	2659	3504	845	24.1
Sunbonnet	8989	R	3133	4140	1007	24.3
Fortuna	1344	R	3843	3822	None	None
TP 49	8991	R	2443	2869	426	14.8
Texas Patna	8321	R	2741	3162	421	13.3
Rexoro	1779	R	2261	2561	300	11.7
LSD 1%					579	
LSD 5%					430	

[a] Accession number, Cereal Crops Research Branch.
[b] R = resistant, MR = moderately resistant, S = susceptible.

susceptible and resistant, are given in Table 2, along with comparable figures from the 1954 test. Yield reductions from white tip in 1955 and 1956 averaged less than 7%, even in the susceptible.

PERSISTENCE OF NEMATODES IN RESISTANT VARIETIES.—Laboratory examination of seed samples of white-tip-resistant varieties from rows or test plots, grown adjacent to or near diseased susceptible varieties, has nearly always shown considerable numbers of nematodes present. Nematodes are also numerous in seed samples from artificially infested plots. However, resistant varieties seldom show typical white-tip symptoms under commercial field conditions. Also, samples from commercial fields of resistant varieties have few, if any, nematodes. As a criterion for resistance, studies were made on the persistence of nematodes in the resistant varieties.

In 1954 and 1955 several resistant varieties were sown within 4×12-ft. sheet-metal forms sunk into the soil, using seed from samples known to contain nematodes. Each plot consisted of 4 rows of a single variety seeded within a metal form to prevent nematodes from being carried from plot to plot in irrigation water. The plots were observed for white tip. At maturity, about 20 panicles were harvested from each plot and the grain samples were examined in the laboratory for live nematodes, using the method previously described (9). Caloro, included as a susceptible control variety, showed white tip each year and fairly large numbers of nematodes in harvested seed. Nematodes, with 2 exceptions, failed to multiply in plants of resistant varieties grown from infested seed; they were not present in harvested seed samples.

TABLE 2.—*Three-year summary of yields for resistant and susceptible classes of rice varieties in white tip tests*

Year	Variety class	Yield (lb./A.)		% reduction
		White tip	Control	
1954	Susceptible	2342	3723	37.1
1954	Resistant	2879	3429	16.0
1955	Susceptible	2778	2922	4.9
1955	Resistant	2421	2459	1.5
1956	Susceptible	3406	3645	6.6
1956	Resistant	2644	2694	1.8
1954–56	Susceptible	2842	3430	17.1
1954–56	Resistant	2648	2861	7.4

VARIETAL RESISTANCE.—For several years, in cooperative studies with rice breeders as a part of a varietal improvement program, many rice varieties and selections have been rated as to reaction to white tip. Disease readings based on natural infection were made each year on the varieties within test plots. In 1953, 1955, and 1956 a large number were also included in special white-tip nurseries. These nurseries were inoculated with laboratory cultures of nematodes as described under the yield tests. The varieties were rated as to disease reaction on the basis of severity of leaf symptoms. The reaction to white tip of the more important rice varieties is shown in Table 3.

DISCUSSION.—For many years, visual ratings of severity of leaf symptoms have been used in classifying rice varieties as to their reaction to white tip. However, the validity of this criterion for resistance has been questioned on the grounds that the plants might simply fail to exhibit foliar symptoms, and seed samples of the resistant varieties from the rice experiment stations generally contain nematodes. Tests showed that, in most cases, white tip reduced yields of susceptible varieties more than those classified as resistant on the basis of foliar symptoms. It may be concluded that ratings based upon leaf symptoms may be used in classifying rice varieties and that white tip will reduce yields of those rated as susceptible.

CROPS RESEARCH DIVISION
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
BEAUMONT, TEXAS

TABLE 3.—*Reaction of rice varieties to white tip*[a]

Variety	C.I. no.[b]	White tip nursery			Natural infection			General reaction
		1953	1955	1956	1954	1955	1956	
Arkrose	8310	VS	S	S	S	S	VS	S
Asahi	8312		R	R	R	MR	R	R
Bluebonnet	8322	MR	R	R		R	R	R
Bluebonnet 50	8990	R	R	R		R	R	R
Blue Rose	2128	VS	S	S	S	S	VS	VS
Blue Rose 41	8317	S			MS		R	MS
Caloro	1561-1	VS	S	S	S	S	VS	S
Calrose	8988	VS	S	S	S	S	VS	VS
Cody	8342	S	S	S		S	S	S
Colusa	1600	VS	S	MS		S	S	S
Century Patna 231	8993	MR	R	R		R	MR	MR
Century 52	9002	MR	R			R		MR
Early Prolific	5883	VS	S	S		VS	S	VS
Fortuna	1344	MR	R	R		R	R	R
Improved Bluebonnet	8992	R	R	R		R	R	R
Kamrose	8314	VS	S		S	VS		S
Lacrosse	8985	VS	S	S		S	S	S
Magnolia	8318	S	S	S		S	S	S
Nato	8998	S	R	MS		MS	MS	MS
Nira	2702	R	R	R		R	R	R
Prelude	8311	VS	S			S		S
Rexark	8644	MS	R	R		MS	MR	MR
Rexoro	1779	R	R	R	R	R	R	R
Sunbonnet	8989		R	R		R	R	R
Texas Patna	8321	R	R	R	R	R	R	R
Toro	9013		R	R		R	R	R
TP 49	8991	R	R	R	R	R	R	R
Zenith	7787	VS	S	S		S	S	S

[a] R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, VS = very susceptible.
[b] Accession number, Cereal Crops Research Branch.

LITERATURE CITED

1. ADAIR, C. R., AND E. M. CRALLEY. 1948. 1947 Arkansas rice yield tests. Arkansas Univ. Fayetteville Agr. Expt. Sta. Rept. Ser. 9, p. 1-21.
2. ADAIR, C. R., AND E. M. CRALLEY. 1950. 1949 rice yield and disease control tests. Arkansas Univ. Fayetteville Agr. Expt. Sta. Rept. Ser. 15, p. 1-20.
3. ADAIR, C. R., AND E. M. CRALLEY. 1952. Rice breeding and disease control studies in Arkansas, 1940 to 1950. Arkansas Univ. Fayetteville Agr. Expt. Sta. Bull. 525, p. 1-23.
4. CRALLEY, E. M. 1952. Control of white tip of rice. Arkansas Farm Research **1** (1): 6.
5. CRALLEY, E. M. 1953. A summary of recent investigations on the control of white tip of rice. Rice Annual, 1953, p. 19-20.
6. JODON, N. E. 1935. Improving rice varieties. Louisiana Rice Expt. Sta. Biennial Rept. 1933-1934, p. 15-18.
7. JODON, N. E., AND D. A. DE LA HOUSSAYE. 1949. Rice varieties for Louisiana. Louisiana Agr. Expt. Sta. Tech. Bull. No. 436, p. 1-15.
8. JONES, J. W., C. R. ADAIR, H. M. BEACHELL, N. E. JODON, AND A. H. WILLIAMS. 1953. Rice varieties and their yields in the United States, 1939-50. U. S. Dept. Agr. Circ. 915, p. 1-29.
9. TODD, E. H., AND J. G. ATKINS. 1958. White tip disease of rice. I. Symptoms, laboratory culture of nematodes and pathogenicity tests. Phytopathology **48**: 632-640.
10. TODD, E. H., AND J. G. ATKINS. 1959. White tip disease of rice. II. Seed treatment studies. Phytopathology **49**: 184-188.

METHODS FOR MICROSCOPIC DETECTION OF APHANOMYCES EUTEICHES AND RHIZOCTONIA SOLANI AND FOR ISOLATION OF RHIZOCTONIA SOLANI ASSOCIATED WITH PLANT DEBRIS[1]

M. G. Boosalis and A. L. Scharen[2]

SUMMARY

Microscopic examination of comminuted plant debris from pea fields indicated that *Aphanomyces euteiches* may overwinter as oospores embedded in dead plant tissues. Soil that grew peas with a high incidence of root rot incited by *A. euteiches* in the field and greenhouse yielded about 9 times as many plant debris particles infested with oospores of the pathogen as did soil with a moderate incidence of the disease. Plant debris fragments infested with *A. euteiches* were not recovered from soil with a low pea root-rot index. Attempts in the laboratory to germinate oospores found in naturally infested plant debris were unsuccessful. Direct microscopic observation also showed that *Rhizoctonia solani* pathogenic to sugar beet seedlings persists in soil in the form of sclerotia on the surface of plant debris particles, and in thick-walled hyphae within such particles. *R. solani* from plant debris screened from field soil was isolated by plating on agar medium to which streptomycin sulphate was added. The percentage of plant debris particles infested with *R. solani* was determined in soil taken from sections of a sugar beet field 7 months after the crop was harvested. In one section of the field there had been a high incidence of crown rot incited by *R. solani*, and in the other area of the field a low incidence of the disease at harvest time. About 8.5% of the plant debris particles from the severely diseased area of the field yielded *R. solani*, whereas only 2% of the particles from soil with a low incidence of crown rot were infested. All of the 35 isolates from the severely diseased area of the field composed a single physiologic race of *R. solani* that caused damping-off and seedling blight of sugar beet; 63% of isolates from the section of the field with a low incidence of crown rot were similar to the above physiologic race of *R. solani*.

The importance of plant debris in soil as a source of potential inoculum for root diseases incited by fungi has been elucidated by several investigators (2, 5). Difficulties of technique are the principal bar to using this information in determining the disease potential of soil and investigating the ecological relationships of root-disease fungi. The more recent techniques (1, 8, 9, 10) used to examine and isolate fungi in soil fail to show the substrate origin of the colonies, especially the fungus flora associated with small fragments of dead plant tissues. Without better methods of ascertaining the presence of potential inoculum associated with plant residue, investigations of root diseases will continue to slight the role of this important source of the pathogen in the development of epidemics. Moreover, if new methods of controlling root diseases are predicated on the assumption that root-infecting fungi in soil reside apart from plant debris, then such measures may fail when used against pathogens that do inhabit plant debris. This investigation was prompted by an urgent need for more effective methods of detecting and isolating pathogenic fungi associated with plant debris.

[1] Accepted for publication November 1, 1958.

Published with the approval of the Director as Paper No. 907, Journal Series, Nebraska Agricultural Experiment Station.

[2] Plant Pathologist and Research Assistant in Plant Pathology, respectively.

The authors are indebted to Dr. K. K. Kaukis and the Green Giant Company for providing soil samples and determining the root rot indices of soils infested with *A. euteiches*. Acknowledgment is also made to Dr. M. L. Schuster, for providing soil infested with *R. solani* and identifying crown-rot of sugar beet caused by *R. solani*.

MATERIALS AND METHODS.—To facilitate the development of methods, it was decided to work first with organisms easily identified by their morphologic features. The root-disease-inciting fungi *Rhizoctonia solani* Kuehn and *Aphanomyces euteiches* Drechs., known to inhabit plant debris, fulfill the requirement (2, 6). The first is easily identified by the morphologic characters of the hyphae, and the second by the morphology of the oospores (3, 6).

Two 50-lb. bulk samples of soil, each consisting of 25 2-lb. random collections, were obtained near Mitchell, Nebraska, in June of 1957, from a commercial barley field on Tripp very fine sandy loam. The soil samples making up each bulk sample were taken from the 1–5 in. layer in one of two areas of the field, each measuring about 30×30 ft. In 1956 one area of this field planted to sugar beet had a high incidence of crown rot incited by *R. solani*, and the other area a low incidence. Moisture content of the two bulk samples was about 35 per cent of water-holding capacity, and pH was about 7.2.

Samples of a Clarion-Webster type of soil naturally infested with *A. euteiches* were collected in the fall of 1957 from 3 11-acre fields of peas near Le Sueur, Minnesota. From a 1/30-acre area in each field a 9-lb. random sample was obtained from the 2–6 in. layer. In 1956 the respective incidences of root rot had been high, moderate, and low; the corresponding moisture contents of the collected soils were 40, 36, and 33 per cent; pH was about 5.5–5.8.

All soil samples were stored in plastic bags at 5°C until used. Changes in moisture and pH during storage were negligible.

A method was developed for detecting both *A. euteiches* and *R. solani* by direct microscopic observation of fungus structures on and in plant debris. A special plating method was designed for isolating *R. solani* from soil. Detailed descriptions of these two methods follow, together with results from experiments using them to determine the potential inoculum of soils naturally infested with *A. euteiches* or *R. solani*.

Direct microscopic observation.—Each of 3 100-g samples of soil was thoroughly suspended in about 2.5 liters of tap water in a metal vessel. The suspension was allowed to stand about 0.5 minute before the supernatant liquid was poured onto a 60-mesh brass sieve set in a frame 5 in. across and 2 in. high. Soil that had settled was resuspended in one liter of tap water and allowed to settle about 0.5 minute and the supernatant liquid passed through the sieve. This procedure was repeated 5–8 times, until the supernatant liquid was relatively free of plant debris particles. Seeds of weeds, large particles of soil and sand, and large pieces of straw and woody plant tissue were removed with forceps from the plant debris screenings.

Next, most of the soil deposited on the screen was removed by holding the screen under running tap water and sharply tapping the frame of the screen against the sink. The remaining plant debris was gently rubbed for about 1.5 minutes and simultaneously rinsed with tap water. The rubbing was done with the tip of a rubber bulb of a medicine dropper that had its glass tube inserted as far as possible into the bulb. Most of the soil intermixed with the plant residue was thereby sufficiently loosened and ground to pass through the screen when rinsed. Little comminution of the plant residue resulted from this first rubbing treatment, and, consequently, nearly all of the plant debris pieces remained on the screen.

The debris particles were next transferred to a 200-mesh screen, and longer and more vigorous rubbing treatment was used to macerate them there and remove soil from the surface of the plant tissues. The best comminution of plant debris particles for microscopic examination resulted from rubbing them 1 minute for *A. euteiches* and 2 minutes for *R. solani*. Plant fragment sizes often varied from 25 to over 700 μ long by 6 to over 100 μ wide. The fragments from soil infested with *R. solani* were more uniform and smaller because they were rubbed longer. The comminuted plant debris, also referred to as the macerated plant debris, now relatively free of soil, was examined microscopically for hyphae and sclerotia of *R. solani* or for oospores of *A. euteiches*.

For microscopic examination, the macerated plant residue from each 100-g sample of field soil was suspended in about 35 ml of tap water, and 5 ml of the suspension dispensed into each of 7 Petri dishes, each containing about 10 ml of 2 per cent water agar. Microscopic observations under the low power field (×100) were made to determine the gross morphology of the oospores of *A. euteiches* and the sclerotia and hyphae of *R. solani*. All fragmented debris particles from each 100-g sample of soil were examined under the binocular dissecting microscope (×45) for determining the number of particles containing A. *euteiches*.

Special plating method.—To isolate *R. solani* from intact nondisinfected plant debris particles from soil, soil samples were screened and prepared as were samples for *A. euteiches*. The unrubbed plant residue screened from 4 100-g samples of soil was spread on separate sheets of filter paper and allowed to dry for about 1 hour. A random sample of 100 plant debris particles from each sample of soil was then transferred to 25 Petri plates, each containing about 15 ml of 2 per cent water agar adjusted with phosphoric acid to pH 4.8. Moisture condensation on the surface of the medium was eliminated by stacking the poured plates upside down for at least 72 hours before using, after which 4 drops of streptomycin sulfate solution (20 mg/ml) were pipetted equidistantly on the medium, about 0.5 in. from the periphery of the plate.

Immediately after the streptomycin sulfate solution diffused into the medium, in about 1 hour, 1 plant debris particle was placed on each of the 4 areas of the medium treated with the antibiotic solution, and the plates were then incubated 5 days at 24°C. By using acidified water agar with a dry surface and by having a relatively high concentration of streptomycin sulfate in the immediate area of the medium in contact with the nondisinfected plant debris, bacterial growth was impeded. Colonies of *R. solani* from plant debris particles were discerned in 4–98 hours, the majority developing in about 48 hours. Species of *Fusarium*, *Trichoderma*, *Pythium*, and several unidentified genera were the first to grow out of many of the plant particles that eventually yielded *R. solani*. These other fungi apparently did not prevent the slower-starting *R. solani* from growing out of its natural substrate.

RESULTS.—*Microscopic detection of A. euteiches.*—Results from microscopic examination of comminuted plant debris particles for *A. euteiches* are given in Table 1 and Fig. 1-A. Oospores embedded in plant debris fragments from 3 commercial fields of pea were morphologically similar to those produced by a pure culture of *A. euteiches* in roots of peas. They were also comparable in size and morphology to those described (6) in the cortex of roots of peas inoculated with a pure culture of *A. euteiches*. Comminution of the plant residue from 100 g of soil with a high index of root rot of peas yielded an average of 55 fragments of plant tissue containing 1–250+ oospores of *A. euteiches*. Most of the infested fragments contained 10–30 sexual spores of the pathogen. On a scale of 1 to 4, from healthy to severe cortical root rot, the root rot index of the soil from this field was assessed at 3.8 in the field and 4 in the greenhouse (Table 1). An average of 6 fragments of plant debris per 100 g of soil from the second field contained oospores of *A. euteiches*, the maximum in a single fragment being

120. The rot indices of this soil were 2.0 and 2.6 in field and greenhouse, respectively (Table 1). None of the 3 100-g replicates of soil with the lowest root-rot indices in the field and greenhouse yielded plant debris fragments infested with *A. euteiches*.

Attempts to germinate oospores of *A. euteiches* found in comminuted plant residue were unsuccessful, although the positive association between number of oospores and degree of severity of root rot of peas grown in the soil from which they came would indicate that they were alive (Table 1). Further research is needed to determine what specific factor(s) are required to stimulate germination of oospores found in plant debris.

Microscopic detection of R. solani.—Hyphae and sclerotia of *R. solani* were observed on comminuted plant residue screened from soil with a high incidence of crown rot of sugar beet and from a second sample of soil with a low incidence of the disease. This method was not used to determine the total number of fragmented plant debris particles infested with *R. solani* per unit weight of soil, because exploratory studies indicated that a more accurate estimate could be made by the special plating method.

Comminuted plant residue from soil with a high incidence of crown rot was used to ascertain whether inoculum of *R. solani* is harbored externally as well as internally in plant debris. This material was also used to determine the gross morphology of the hyphae and sclerotia of *R. solani* associated with plant residue. Viability of hyphae and sclerotia was determined by placing random samples of infested comminuted plant debris fragments in 4 drops of tap water on glass slides, one particle per drop of water. The glass slides were then transferred to Petri plates containing moist filter paper and incubated 96 hours at 24°C.

TABLE 1.—*Relation between fragmented plant debris particles containing oospores of Aphanomyces euteiches and severity of root rot of peas tested in field and greenhouse*[a]

Root rot index of pea[b]		Replication No.	No. infested plant debris particles per 100 g soil[c]
Field	Greenhouse		
		1	47
3.8	4.0	2	65
		3	53
		Average	55
		1	8
2.0	2.6	2	3
		3	7
		Average	6
1.0	1.5	1–3[d]	Average 0

[a] Soil was from 3 commercial fields of pea near LeSueur, Minnesota.

[b] Each index is the mean rating of 5 plants in each of 3 flats. The roots of plants were scored on a scale of 1 to 4: 1, healthy roots; 2, superficial lesions; 3, lesions extending to surface layers of cortex; 4, very severe cortical rot, resulting in severe stunting or death of the plant.

[c] An average based on number of fragments infested with oospores of *A. euteiches* from 3 replications of soil. Total number of fragments in each replicate was not counted.

[d] No oospores were discerned in fragments from 3 samples of soil.

From 3 100-g samples of soil a random sample of 500 fragmented plant debris particles infested with *R. solani* was microscopically examined for hyphae and sclerotia of this fungus. Sclerotia were found on about 74 per cent of the *Rhizoctonia*-infested plant particles (Fig. 1-B). Mycelia morphologically similar to those of *R. solani* were discerned on the surface of about 68 per cent of the *Rhizoctonia*-infested plant residue fragments (Fig. 1-C). Low counts of debris particles with external hyphae and sclerotia undoubtedly were due to the fact that the rubbing treatment used to macerate plant debris particles dislodged many of these fungus structures. The subepidermal tissues of 44 per cent of the infested plant debris fragments were invaded by hyphae of *R. solani* (Fig. 1-D). About 60 per cent of the comminuted infested tissues examined had internal and external hyphae, and sclerotia. Less than 10 per cent of the fragmented plant residue contained only one of the three types of fungus structures. Results were comparable when the experiment was repeated 4 times.

As shown in Fig. 1-C, the external dark-brown filamentous hyphae of *R. solani* on the surface of plant debris branched considerably and appeared devoid of protoplasm. Typical *Rhizoctonia*-type constrictions were evident at the point of union of the hyphal branches. Of the 500 external hyphae examined, none resumed growth when the infested plant debris was incubated on slides 4 days at 24°C.

The dark-brown to black sclerotia varied in size from minute cell groups less than 10 μ in diameter to large, stroma-like bodies that extended over the entire surface of plant debris fragments measuring over 500 μ in length. The smaller, discrete type of sclerotium was spherical or irregular, being rather flattened, and somewhat rough on the surface. Of the 350 discrete and stromatic sclerotia on the surface of 300 plant debris particles, about 44 per cent germinated, a single one producing as many as 80 germ tubes (Fig. 1-B). Most of the sclerotia in contact with tap water germinated after 24 hours at 24°C; for the other sclerotia the times varied from 3 to 96 hours. Of 70 isolates from sclerotia produced on 70 plant debris fragments on glass slides, all were similar in morphology and pathogenicity in causing damping-off and seedling blight on sugar beet.

The relatively thick-walled hyphae within plant debris tissue were dark-brown and irregularly branched (Fig. 1-D). Protoplasm, together with globular material, was present in many of the hyphal cells. The majority of these internal-type hyphae were composed of long, moniliform, chain-like aggregations of cells, as described by Duggar (3), many producing germ tubes like the ones he saw. About 35 per cent of 200 hyphae embedded in 190 plant debris fragments produced new growth. Fungus colonies established from 60 of the internal-type hyphae were identified as *R. solani*. Moreover, these isolates were similar in mor-

phology and pathogenicity to those from sclerotia. The results indicate that a pathogenic strain of *R. solani* causing damping-off and seedling blight of sugar beet may persist in soil for at least 7 months as hyphae in dead plant tissue and as sclerotia on the surface of plant debris.

Isolation of R. solani by plating.—Soil from the area of the field with a high incidence of crown rot

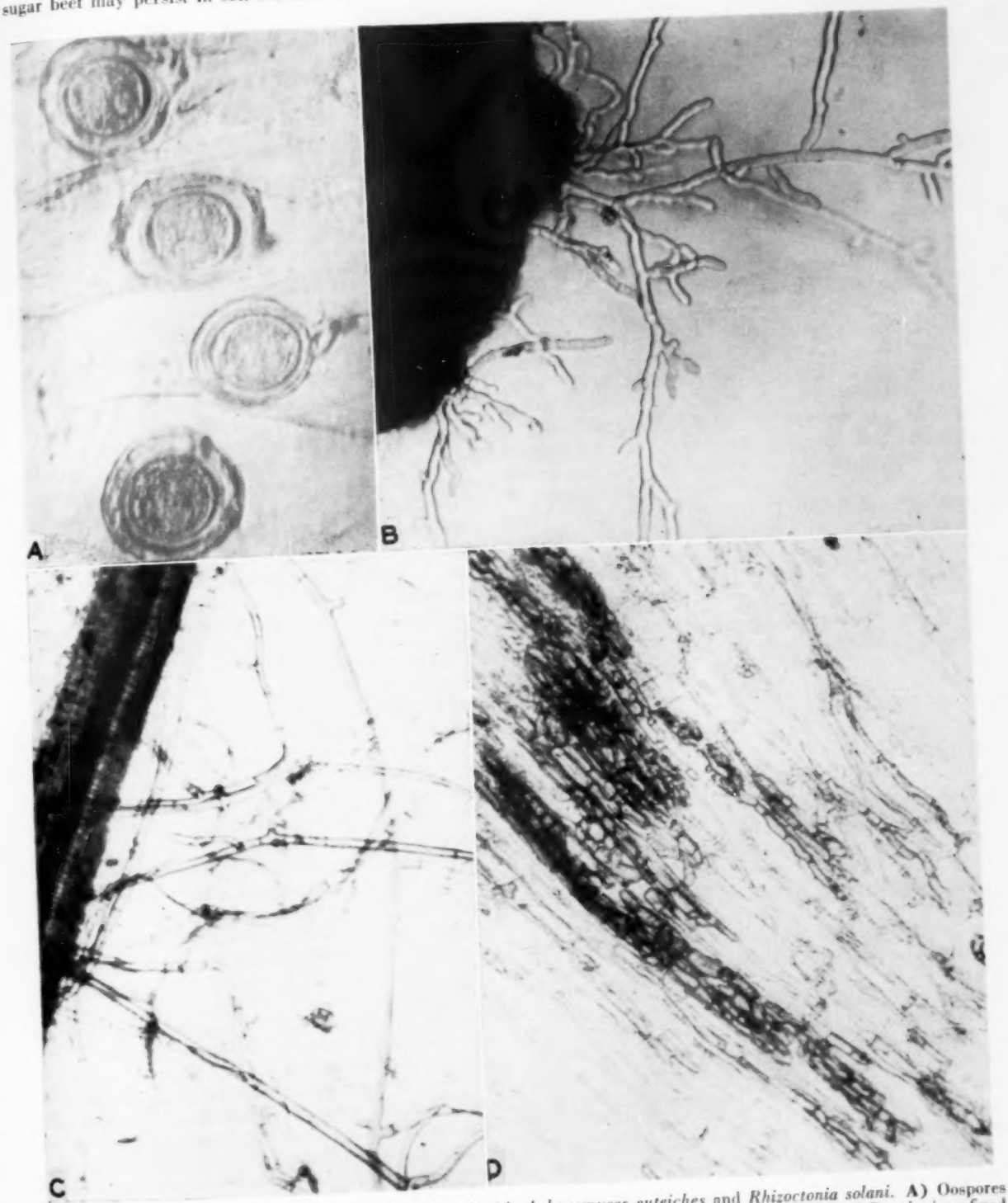


Fig. 1. Comminuted plant debris naturally infected with *Aphanomyces euteiches* and *Rhizoctonia solani*. **A)** Oospores embedded in plant debris (×880). **B)** Germinating sclerotium of *R. solani* (×230). **C)** Hyphae of *R. solani* confined to surface of plant debris (×230). **D)** Thick-walled hyphae of *R. solani* embedded in plant debris (×230).

of sugar beet contained a relatively high percentage of plant residue infested with *R. solani*. Viable inoculum of *R. solani* was harbored by 35 (8.5 per cent) of the 400 plant debris particles screened from 4 100-g replicated samples of soil (Table 2). The number of plant debris particles infested with *R. solani* in each replicate was fairly consistent, with a minimum of 7 and a maximum of 10 (Table 2). The 35 isolates of *R. solani* from the plant debris particles were similar in the color and morphology of colonies and sclerotia when grown 18 days at 24°C on malt agar, corn-meal agar, Lima-bean agar, Czapek Dox agar, potato-dextrose agar, and V-8 juice agar. Furthermore, the gross morphological characteristics of these isolates grown on the six media were like those of a known strain of *R. solani* that incites seedling blight and crown rot of sugar beet. Greenhouse tests with sterilized soil kept at 27°C indicated that the 35 isolates of *R. solani* were moderately pathogenic in causing seedling blight and damping-off of sugar beet variety Great Western 359-53R. It is conceivable that some of the isolates of *R. solani* shown to be moderately pathogenic to germinating seeds and seedlings of sugar beet are not equally pathogenic on the roots and beet of older plants, but this was not determined. The similarity of the 35 isolates of *R. solani* in cultural characters and pathogenicity to seedlings of sugar beet indicates that these isolates make up a single physiologic race, or at least closely related races, of *R. solani*.

The amount of potential inoculum of *R. solani* contained in this soil 7 months after the crop of sugar beet was harvested was tremendous. Based on the average number of plant debris particles counted in 40 1-g random samples of soil, it was estimated that 100 g of soil from the 1–5 in. layer contained about 80,000 plant debris particles. Thus, 100 g of such a soil harbors about 6800 such particles infested with *R. solani*.

The soil from the area of the field with a low incidence of crown rot of sugar beet yielded only 8 plant debris particles infested with *R. solani* out of 400 (Table 2), the range being 0–3 per sample. On the basis of cultural characteristics of the colony and pathogenicity, the 8 isolates of *R. solani* composed 2 physiologic races. Five of the isolates were identical to the race causing damping-off and seedling blight of sugar beet. The remaining 3 were similar in cultural characteristics on the 6 synthetic media and in pathogenicity on potato stems to a known strain of *R. solani* that causes stem rot of potato. These 3 isolates were not pathogenic to sugar beet seedlings. It was estimated that 100 g of soil from the area of the field with a low incidence of crown rot contained about 30,500 plant debris particles, 600 of which were infested with *R. solani*. Of the 600, 378 would yield the physiologic race pathogenic to sugar beet seedlings, and the remaining 222 the physiologic race pathogenic to potato stems. The relatively small number of plant debris particles recovered from this soil compared to the heavily diseased area can be explained by the fact that the latter soil had considerably more deteriorated beets at harvest time.

TABLE 2.—*Percentage of plant debris particles infested with Rhizoctonia solani from soil obtained from 2 contrasting areas of a field*

Source of soil	Replication number[a]	% of plant particles yielding *R. solani*[b]
Area with high incidence of crown rot of sugar beet	1	7
	2	10
	3	9
	4	9
	Average	8.5
Area with low incidence of crown rot of sugar beet	1	3
	2	3
	3	0
	4	2
	Average	2

[a] Each replication consisted of 100 g of soil.
[b] Percentage is based on number of plant debris particles yielding *R. solani* out of 100.

Standard soil dilution plate methods were unsatisfactory for isolating *R. solani* from the two collections of field soil. A final soil dilution of 1:5000 was prepared as outlined by Fred and Waksman (4). By means of 1-ml bacteriological pipette, samples of 1 ml of the final soil dilution were plated on Martin's peptone-dextrose agar with rose bengal and streptomycin sulfate, on Warcup's modified Czapek Dox agar, and on acidified water agar supplemented with streptomycin sulfate as described in this paper (7, 9). Each dilution sample plated by this means contained either a few or no plant debris particles. Two hundred plates of each of the 3 basal media were prepared for each collection of field soil. The plates were incubated 5 days, half at 21°C and half at 28°C, and examined daily for the presence of *R. solani*. This method of isolation failed to yield *R. solani* on any of the plates, although the attempt was repeated 3 times within 3 weeks.

Results from this cursory study indicated that the soil with a high amount of crown rot of sugar beet contains 18 times as many plant debris particles infested with *R. solani* pathogenic to sugar beet seedlings as does soil with a low incidence of the disease. It would appear that plant debris infested with *R. solani* constituted the primary source of inoculum for both areas of the field 7 months after the sugar beet crop was harvested.

DISCUSSION.—The excellent agreement between the high number of plant debris fragments infested with oospores of *A. euteiches* and the severe root rot of peas in soil from which the plant residue was obtained suggests that this pathogen overwinters by means of sexual spores in dead plant tissues. Further research is needed to determine what factor(s) stimulate germination of oospores and to establish the mode of germination and longevity of these spores in naturally infested plant tissue. Heretofore, such studies have been hampered by a lack of techniques for

detecting *A. euteiches* in its natural habitat. By means of the direct microscopic method for observing oospores embedded in plant debris fragments, it is possible to investigate more systematically many problems relative to the ecology and epidemiology of root rot of peas incited by *A. euteiches*. Studies now in progress by Scharen indicate that oospores of this pathogen in plant debris particles germinate to produce zoospores, as described by Jones and Drechsler (6) from studies with pure cultures of *A. euteiches*. Results of this study will be published elsewhere.

Unpublished data by Boosalis and Scharen indicate that it may be feasible to predict the disease potential of a field with respect to root rot of peas caused by *A. euteiches* by the direct microscopic method. Before such predictions can be made with confidence, however, the method will have to be further refined. The minimum number of plant debris fragments infested with *A. euteiches* necessary to cause a high, medium, or low root-rot index of peas should be established per unit weight of soil before this method is used to evaluate the disease potential of fields.

By means of the direct microscopic observation technique, it was shown that *R. solani* inciting damping-off and seedling blight of sugar beet persists in soil as hyphae and sclerotia within and on plant debris, respectively. Lots of the *Rhizoctonia*-infested plant debris particles contained both sclerotia and hyphae, whereas other lots harbored only one of these fungus structures. Germination of the irregularly branched, dark-brown hyphae embedded in dead plant tissues occurs from an inner one of several connected cells, as described by Duggar (3) from studies on pure cultures of the pathogen. To the authors' knowledge, this is the first report on the manner of germination of hyphae of *R. solani* produced within naturally infested plant debris particles. The number of germ tubes developing from a sclerotium varied from 2 to as many as 80. It should be noted that all of the observed hyphae of this fungus confined to the surface of plant debris were devoid of protoplasm, and none of them germinated. These results lead to the conclusion that the most effective methods for reducing inoculum of *R. solani* in soil are those that are deleterious to the sclerotia harbored on the surface of plant debris particles and to the hyphae within dead plant tissues. In this connection, investigations are now in progress to determine the effect of soil fumigants on the survival of hyphae and sclerotia of *R. solani* associated with plant debris particles.

The direct microscopic observation method was not satisfactory for establishing the potential inoculum of *R. solani* contained by plant debris particles. The principal disadvantage of this method is that it dislodges all of the sclerotia from some of the plant debris tissues, thereby introducing a serious error in estimating the number of substrate sites infested with *R. solani*. For this reason, then, a more reliable method was designed for ascertaining the percentage of plant debris particles in soil infested with *R. solani*.

A modification of the plating method used for isolating fungus pathogens from diseased plant tissues was developed for isolating *R. solani* from unmacerated plant residue from soil. A detailed account of this method is given in the text. The majority of *R. solani* colonies established on the medium from plant residue were free of bacterial contamination. Other fungi, such as *Trichoderma* spp., *Fusarium* spp., and *Pythium* spp. developing on the medium before or concurrently with *R. solani*, did not inhibit the rapid growth of the latter.

The relative number of plant debris particles infested with *R. solani* from two areas of a commercial field of sugar beets was determined by the above method about 7 months after the crop was harvested. About 8.5 per cent of the plant residue from soil with a high incidence of crown rot of sugar beet incited by *R. solani* yielded *R. solani* (Table 2). All of the isolates of *R. solani* from this source of plant debris particles were of one physiologic race. This particular race was moderately pathogenic in causing damping-off and seedling blight of sugar beet. The pathogenicity of this race on beets and roots of older plants was not determined. It was estimated that 100 g of soil from a 1–5 in. layer in the severely diseased area of the field contained about 6800 plant debris particles infested with this one particular race of *R. solani*. On the other hand, about 2 per cent of the plant debris particles from the area of the field with a low incidence of crown rot of sugar beet yielded *R. solani*. About 63 per cent of the isolates were identical to the above-described physiologic race of *R. solani*. The remaining 37 per cent of the isolates were pathogenic to potato stems, but not to sugar beet seedlings. A 100-g sample of soil from the lightly infested area of the field was calculated to have 378 plant debris particles infested with the physiologic race of *R. solani* pathogenic to sugar beet. Thus the 1–5 in. layer of soil with a high incidence of crown rot harbored about 18 times as much potential inoculum of *R. solani* as did soil with a low incidence of the disease. In this connection, it should be stated that the severely diseased area of the field contained many more deteriorated beets than did the area with a low incidence of crown rot. This undoubtedly accounts for the fact that the section of the field with a high incidence of crown rot yielded twice as many plant debris particles as soil with a low level of the disease. Isolation of only two physiologic races of *R. solani* from plant residue does not preclude the possibility that other races of this fungus are associated with plant debris particles in soil. Failure to isolate additional races may be ascribed to the relatively small samples of plant debris particles used in this study. Notwithstanding the inadequacy of the samples for isolating less prevalent races, the results clearly show that the physiologic race of *R. solani* causing damping-off and seedling blight of sugar beet was predominantly associated with plant residue from diseased areas of the field 7 months after the crop

was harvested. Moreover, the positive correlation between the number of propagules in soil to disease intensity of crown rot of sugar beet and root rot of peas incited by *R. solani* and *A. euteiches*, respectively, is in accord with results from studies with other root diseases (5).

In contrast to the above results with the modified plating method, colonies of *R. solani* were not recorded on any occasion on dilution plates prepared from soil from the 2 areas of the field. Exclusion of nearly all plant debris particles by the common dilution method for plating fungi from soil vitiates the efficacy of such a method for determining the potential inoculum of *R. solani* and other fungi associated with plant residue.

Whether or not the modified plating method can be used to determine the disease potential of a field with respect to root rot of sugar beet caused by *R. solani* is a question for further investigation.

Department of Plant Pathology
Nebraska Agricultural Experiment Station
Lincoln

LITERATURE CITED

1. Chesters, C. G. C. 1948. A contribution to the study of fungi in the soil. Brit. Mycol. Soc. Trans. **30:** 100-117.
2. Chesters, C. G. C. 1949. Concerning fungi inhabiting soil. Brit. Mycol. Soc. Trans. **32:** 197-216.
3. Duggar, B. M., and F. C. Stewart. 1901. The sterile fungus Rhizoctonia as a cause of plant diseases in America. N. Y. State Agr. Expt. Sta., Geneva, N. Y., Bull. 186. 30 p.
4. Fred, E. D., and S. A. Waksman. 1928. Laboratory manual of general microbiology. McGraw-Hill Book Co., N. Y. 145 p.
5. Garrett, S. D. 1956. Biology of root-infecting fungi. Univ. Press, Cambridge, England. 292 p.
6. Jones, F. R., and C. Drechsler. 1925. Root rot of peas in the United States caused by Aphanomyces euteiches (N.SP.). J. Agr. Research **30:** 293-325.
7. Martin, J. P. 1950. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci. **69:** 215-232.
8. Thornton, R. H. 1952. The screened immersion plate: a method of isolating soil micro-organisms. Research (London) **5:** 190-191.
9. Warcup, J. H. 1950. The soil-plate method for isolation of fungi from soil. Nature **166:** 117-118.
10. Warcup, J. H. 1955. Isolation of fungi from hyphae present in soil. Nature **175:** 953-954.

MULTIPLE VIRUS DISEASE RESISTANCE IN A STRAIN OF CAPSICUM ANNUUM[1]

A. A. Cook and C. W. Anderson

SUMMARY

A strain of *Capsicum annuum*, designated P11, was found resistant to strains of the tobacco mosaic, tobacco etch, and potato Y viruses. Plants of this pepper reacted in a hypersensitive manner to most strains of mosaic virus, were tolerant of most strains of etch virus, and were unaffected by all Y virus strains used. Graft inoculations established that virus Y can move through P11 stems, but failed to demonstrate that the virus can multiply within the plants. The reactions of P11 plants to the viruses used were compared with those of *C. frutescens* var. Tabasco and *C. annuum* var. S.C. 46252. Plants of the P11 strain were susceptible to the alfalfa mosaic, cucumber mosaic, and aster ringspot viruses.

Bell pepper plants (*Capsicum annuum* L.) (11) are susceptible to many viruses, but only 5 have been isolated from naturally infected plants in Florida (1). These are the viruses of TMV (tobacco mosaic), TEV (tobacco etch), PVY (potato Y), CMV (cucumber mosaic), and ARSV (aster ringspot). The first 3 infect pepper plants throughout central Florida (1), and the third is very destructive in south Florida (10).

Holmes (5, 6) found TMV resistance in *C. frutescens* L. var. Tabasco, and in *C. annuum* vars. Long Red Cayenne and Sunnybrook Cheese. McKinney (7) first reported resistance to TEV in *C. annuum*, and Greenleaf (4) later studied resistance in both species. Resistance to PVY in *C. annuum* was first reported by Roque and Adsuar (8).

During preliminary tests, a strain of *C. annuum* (hereinafter designated P11) appeared resistant to TMV, TEV, and PVY. Following is an account of more extensive tests with plants derived from a single P11 plant.

Materials and methods.—The TMV strains used were: American Type Culture Collection isolate #2 (TMV-A); an isolate of the "yellow pod" type (1) obtained from a naturally infected *Physalis elliottii* Kze. plant (TMV-P); an isolate from naturally infected tobacco (TMV-T); and McKinney's latent strain (TMV-L).

The TEV isolates used were: "E" strain supplied by S. P. Doolittle (TEV-E); tobacco severe etch virus furnished by F. O. Holmes (TEV-S); and isolates TEV-754, 763, 764, 792, and 793 obtained from H. H. McKinney.[2]

The following PVY strains were tested: the isolate of Conover and Fulton (2), supplied by M. K. Corbett (PVY-N); an isolate typical of the PVY strains

[1] Accepted for publication November 5, 1958.
Florida Agricultural Experiment Station Journal Series, No. 791.

[2] In a personal communication dated January 3, 1958, McKinney said that these numbers signify dehydrated stock cultures of isolates from different sources; that, of the 12 he has tested, these 5 isolates are the only ones that infected pepper variety S.C. 46252; and that No. 763 (ATCC No. 19, Holmes' severe strain) and No. 764 (Greenleaf's Alabama No. 2 isolate) infected this pepper with greater regularity than did No. 754, 792, or 793.

TABLE 1.—*Reaction of P11 pepper plants to mechanical inoculation with strains of six viruses*

Virus and strain	Plants showing symptoms[a]	Symptoms[b]	Recovery attempts from: Inoculated parts	Recovery attempts from: Uninoculated parts	Days observed
TMV-A	47/47	L			28–52[d]
TMV-P	24/24	L			28–52
TMV-L	10/10	m			21*
TMV-T	22/22	L		2/3[c]	28–52
TEV-E	0/21	S	0/4[c]	0/8	31–95*
TEV-S	0/19	S	0/4	0/11	28–68*
TEV-754	0/5	S	0/2	0/5	32–38*
TEV-763	7/8	m	1/2	6/6	32–43*
TEV-764	0/9	S	2/4	3/8	30–49*
TEV-792	0/4	S	0/1	0/4	38–40*
TEV-793	0/4	S	0/1	0/4	39–40*
PVY-C	0/29	S	0/3	0/10	34–95*
PVY-N	0/8	S	0/4	0/8	35–104*
PVY-P	0/9	S	0/3	0/9	35–95*
PVY-S	0/6	S		0/6	50–104*
PVY-T	0/8	S			30–37
AMV	4/4	M		2/2	21*
CMV	4/4	M		2/3	14–21*
ARSV	4/4	Y		2/2	21*

[a] Number of plants with visible symptoms / number of plants inoculated.

[b] L = necrotic lesions on inoculated leaves plus necrotic lesions on stems and uninoculated leaves of some plants; M = severe mottle of uninoculated leaves plus necrosis of stems and both inoculated and uninoculated leaves; Y = local and systemic yellow ring and oak-leaf patterns; m = mild mottle of uninoculated leaves (with some necrosis in the case of TMV-L); S = no symptoms.

[c] Number of plants that yielded virus in recovery inoculations / number of plants from which recovery was attempted.

[d] Number of days plants observed after inoculation. Starred (*) numbers indicate days between inoculation and attempted recovery of virus.

that infect pepper in central Florida (1) (PVY-C); a strain from naturally infected pepper in south Florida, secured by Simons (9) (PVY-S); a potato isolate obtained from H. H. McKinney (PVY-P); and a strain recovered from Florida-grown tobacco (PVY-T). With the possible exception of PVY-C and PVY-S, all strains were distinctly different from each other, and none induced severe systemic necrosis of *C. annuum* var. Large Bell Hot (unpublished information).

M. K. Corbett supplied the strain of AMV (alfalfa mosaic virus), and the CMV and ARSV isolates were on hand.

The carborundum method of mechanical inoculation was used to inoculate pepper plants grown in greenhouses. Infectivity of the inoculum was checked by inoculating susceptible plants. The reactions of Tabasco plants (3, 5, 9) were used to identify most viruses in recovery tests, but *Nicotiana tabacum* L. and *N. glutinosa* L. plants were used as needed.

Cleft grafts were used to inoculate some plants with strains of TEV and PVY. Susceptible pepper scions were grafted onto P11 stocks in some cases, and reciprocal grafts were used in others. The grafted plants were allowed to grow before their susceptible parts were mechanically inoculated, except that previously infected susceptible material was used with PVY-T.

Cleft grafts were also used in attempts to recover virus from graft-inoculated plants. In all such tests, a scion was removed from the P11 portion of the graft-inoculated plant and grafted onto a healthy susceptible pepper stock. In addition, in some recovery tests, a scion from a healthy susceptible pepper plant was grafted onto the P11 part of the graft-inoculated plant. The incubation periods observed among check grafts of infected susceptible scions on healthy susceptible stocks showed that the stocks onto which P11 scions were grafted were kept longer than needed for symptom development.

Because the susceptible peppers used in the graft recovery tests reacted to TEV and PVY in similar manner, infected recovery material was mechanically inoculated to differential hosts (usually Tabasco pepper). But, since typical susceptible varieties (e.g., California Wonder and Tabasco) do not react to mechanical inoculation with PVY-T, other susceptible varieties were used in graft transmission and recovery tests with this virus.

Although the tests reported herein were carried out over a considerable range of environmental conditions, most inoculations were during the cooler months. In other experiments the isolates readily infected susceptible pepper varieties throughout the year. Although most TMV strains sometimes caused severe systemic necrosis on various varieties in summer, TMV-L produced mottle on plants of both P11 and Tabasco throughout the year.

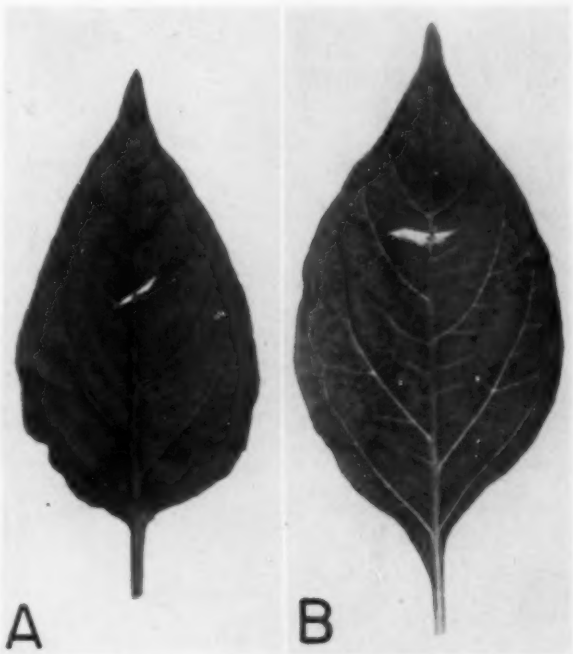


Fig. 1. Localized necrosis of inoculated pepper leaves 4 days after mechanical inoculation with TMV-T. **A)** P11 leaf. **B)** Tabasco leaf (about ×1.2).

RESULTS.—*Tobacco mosaic virus.*—Although P11 plants infected with TMV-L were mottled, the other 3 strains of TMV produced necrotic lesions on inoculated leaves (Table 1). These lesions were slightly larger and less distinct than those induced by the same strains on Tabasco plants (Fig. 1). Necrotic lesions later developed on uninoculated leaves and/or stems of some P11 plants (Fig. 2-A). After the affected leaves dropped, there was no further evidence of systemic spread. However, 1 plant inoculated in April and kept through the warm weather of May died about 50 days after inoculation. Systemic spread was occasionally evidenced on Tabasco plants (Fig. 2-B). Fig. 2-C illustrates a case in which the Tabasco stem was girdled, but axillary growth was initiated below the necrotic area, and the plant survived. Both Tabasco and P11 plants responded to TMV-L with mild mottle and indistinct necrotic patterns. The necrotic patterns are different from those of the other 3 strains on both varieties.

Tobacco etch virus.—Of 7 TEV strains used, only TEV-763 induced visible symptoms on mechanically inoculated P11 plants, and only TEV-764 incited symptomless systemic infection (Table 1). TEV-764 also failed to induce symptoms on graft-inoculated plants, but TEV-E and TEV-S caused mild mottle on some of such plants (Table 2). Susceptible pepper scions grafted onto graft-inoculated P11 plants invariably became infected, whether or not the graft-inoculated P11 plants exhibited symptoms. However, grafting P11 scions from the graft-inoculated plants onto healthy susceptible pepper stocks, or inoculating Tabasco seedlings with sap from graft-inoculated P11 plants, transmitted TEV-E and TEV-S only when symptoms were present on the graft-inoculated plants, with 1 exception. No symptoms appeared on P11 plants graft-inoculated with TEV-E or TEV-S until 46–135 days after inoculation, and then only on some of the plants cut back to stimulate axillary growth.

Similar, though less extensive, tests were conducted with the TEV-resistant pepper S.C. 46252 (4, 7). The results were similar to those obtained with P11, except as follows: 1) TEV-763 caused somewhat stronger symptoms on mechanically inoculated plants; 2) TEV-764 induced symptoms (mild mottle) on some mechanically inoculated plants; 3) TEV strains S and 792 were sometimes recovered from uninoculated parts of symptomless mechanically inoculated plants.

Potato virus Y.—Inoculations with 5 PVY isolates induced no symptoms on P11 plants even though some

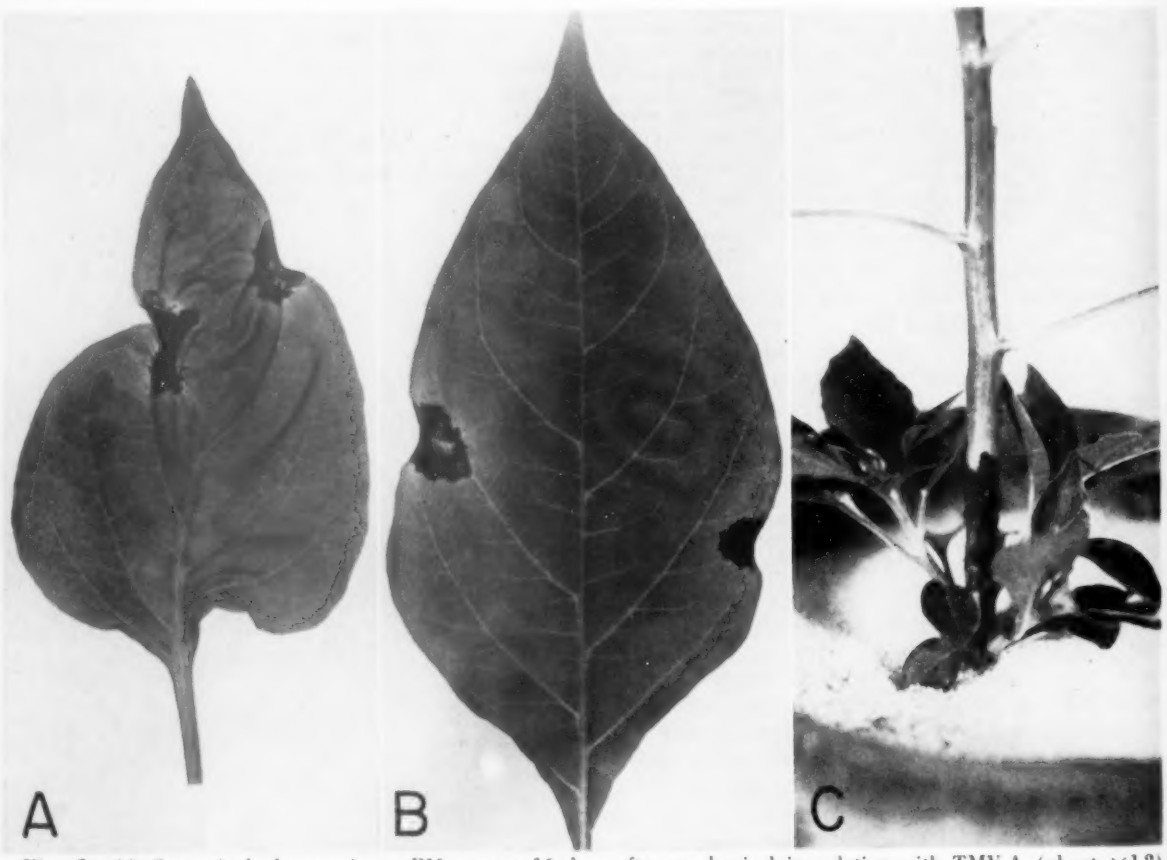


Fig. 2. A) Systemic leaf necrosis on P11 pepper 16 days after mechanical inoculation with TMV-A (about ×1.2). **B)** Systemic leaf necrosis on Tabasco pepper 51 days after mechanical inoculation with TMV-A (about ×1.2). **C)** Stem necrosis on Tabasco pepper 53 days after mechanical inoculation with TMV-A. Note axillary growth below necrosis (about ×1).

TABLE 2.—*Reaction of P11 pepper plants to graft inoculation with the tobacco etch and potato Y viruses*

Virus and strain	Days observed	Plants showing symptoms	Results of recovery attempts: Mechanical recovery: Attempts	Mechanical recovery: Days	Graft recovery: Sus.[a] GP	Graft recovery: P11[b] Sus.	Graft recovery: Days
TEV-E	82–146	3/8[c]	3/8[d]	52–136[e]	3/3[d]	1/3[d]	60–126[e]
TEV-S	60–110	2/8	2/8	46–102	2/2	1/3	60–76
TEV-754	42–82	1/4	1/4	34–54			
TEV-763	37–54	3/4	3/4	37–54			
TEV-764	62–103	0/2	1/2	51–93	1/1	1/1	68
PVY-C	82–146	0/9	0/7	58–125	3/4	0/5	69–97
PVY-N	82–160	0/8	0/6	58–151	2/2	0/4	60–108
PVY-P	82–153	0/7	0/7	115–145	3/3	0/3	76–126
PVY-S	82–119	0/8	0/6	62–102	2/2	1/4	68–91
PVY-T	91–96	0/2			0/1	0/2	61

[a] Scion of susceptible pepper grafted onto P11 graft-inoculated plant.
[b] Scion taken from graft-inoculated P11 plant and grafted onto healthy susceptible pepper stock.
[c] Number of plants with symptoms / number of plants inoculated.
[d] Number of plants from which virus was recovered / number of plants from which recovery was attempted.
[e] Days between inoculation of plants and attempted recovery by the method indicated.

plants were observed for 5 months after inoculation (Tables 1 and 2). Attempts uniformly failed to recover virus from inoculated or uninoculated leaves of mechanically inoculated plants. Attempts to recover PVY from graft-inoculated plants were usually successful when susceptible pepper scions were grafted onto the P11 plants, but unsuccessful (with a single exception) when scions from the graft-inoculated P11 plants were grafted onto healthy susceptible pepper stocks. A second attempt to recover virus from the exceptional plant was negative. Attempts to recover PVY from graft-inoculated plants by mechanical inoculation were uniformly negative. Similar, though less extensive, tests with plants of the TEV-resistant pepper S.C. 46252 gave no evidence that this variety is susceptible to PVY.

Other viruses.—Mechanical inoculations with AMV, CMV, and ARSV proved that P11 plants are susceptible to all 3 viruses (Table 1). Plants infected with the first 2 viruses developed severe leaf mottle and necrotic patterns on leaves and stems. ARSV-infected plants had yellow ring and oak-leaf patterns.

DISCUSSION.—Holmes established the existence of resistance (hypersensitivity) to TMV in the genus *Capsicum*. Later workers added to the list of *Capsicum* species and varieties known to be resistant, and incorporated resistance into some commercial varieties. The writers are not aware that resistance comparable to that of Tabasco has been reported from *C. annuum*. Although some evidence was obtained that the reaction of P11 plants to sap and graft inoculation is similar to that of Tabasco (unreported studies), a definite conclusion cannot be reached without further study.

The degree of resistance of P11 to TEV depended largely on the virus strain used. P11 plants were resistant (tolerant) to 5 strains of TEV, but resistance to 2 others was less evident. Present information suggests that the reactions of P11 and S.C. 46252 to TEV strains are similar, but possibly not identical.

Since no PVY strain used in this study was identical with that studied by Roque and Adsuar, the resistance of P11 cannot be compared with that reported by those authors. From the results herein recorded, it is evident that PVY can move through P11 stems, but it was not demonstrated that PVY can multiply within P11 plants.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF FLORIDA
GAINESVILLE, FLORIDA

LITERATURE CITED

1. ANDERSON, C. W., AND M. K. CORBETT. 1957. Virus diseases of peppers in central Florida. Survey results 1955. Plant Disease Reptr. **41**: 143-147.
2. CONOVER, R. A., AND R. W. FULTON. 1953. Occurrence of potato Y virus on tomatoes in Florida. Plant Disease Reptr. **37**: 460-462.
3. GREENLEAF, W. H. 1953. Effects of tobacco-etch virus on peppers (Capsicum sp.). Phytopathology **43**: 564-570.
4. GREENLEAF, W. H. 1956. Inheritance of resistance to tobacco-etch virus in Capsicum frutescens and in Capsicum annuum. Phytopathology **46**: 371-375.
5. HOLMES, F. O. 1934. Inheritance of ability to localize tobacco-mosaic virus. Phytopathology **24**: 984-1002.
6. HOLMES, F. O. 1937. Inheritance of resistance to tobacco-mosaic disease in the pepper. Phytopathology **27**: 637-642.
7. MCKINNEY, H. H. 1952. Two strains of tobacco-mosaic virus, one of which is seed-borne in an etch-immune pungent pepper. Plant Disease Reptr. **36**: 184-187.
8. ROQUE, A., AND J. ADSUAR. 1941. Studies on the mosaic of peppers (Capsicum frutescens) in Puerto Rico. J. Agr. Univ. Puerto Rico **25**: 40-50.
9. SIMONS, J. N. 1956. The pepper veinbanding mosaic virus in the Everglades area of south Florida. Phytopathology **46**: 53-57.
10. SIMONS, J. N., R. A. CONOVER, AND J. M. WALTER. 1956. Correlation of occurrence of potato virus Y with areas of potato production in Florida. Plant Disease Reptr. **40**: 531-533.
11. SMITH, P. G., AND C. B. HEISER, JR. 1951. Taxonomic and genetic studies on the cultivated peppers, Capsicum annuum L. and C. frutescens L. Am. J. Botany **38**: 362-368.

LOCUS, TIME, AND MODE OF ACTION OF WESTERN X-LITTLE CHERRY VIRUS IN SWEET CHERRY TREES IN OREGON[1]

Leo E. Jones[2]

SUMMARY

Western X-little cherry virus induced diagnostic fruit symptoms in many sweet cherry trees that were growing vigorously and had normal-appearing vegetative tissues. Consistent symptoms were restricted to the fruits and seeds at harvest time. The locus of virus-induced changes was within each fruit and seed; the time was during the pre-harvest flush of growth; the mode was to inhibit enlargement of cells in the mesocarp and to prevent the accumulation of oil within developing embryos. Characteristic variations in degree of symptom expression by individual fruits could be caused by localized effects of the virus within each fruit and seed.

INTRODUCTION.—Western X-little cherry disease is caused by one or more strains of that group of viruses which induce western X-disease in sweet cherries, sour cherries, chokecherries, and peaches. The diverse symptoms caused by this group of viruses are summarized by Reeves et al (11). The symptoms vary with the strain of virus, the host, geographical location, and, in cherries, the rootstock. The relationships between symptoms and the locus, time, and mode of action for each strain of the virus under each set of conditions must be known if the disease is to be understood.

In Oregon, many sweet cherry trees severely affected by western X-little cherry virus produced vigorous new growth and normal-appearing foliage for many years after infection. These trees appeared to be growing on mazzard roots, but the rootstock could not always be positively identified. Reliable diagnostic symptoms were expressed only at harvest time in some of the fruits. Some mature fruits were severely affected, some were intermediate, and the rest were symptomless. Severely diseased fruits were small, conical, and pale (Fig. 1, 2). These three fruit types on diseased trees commonly blended one into the other, and the types were mixed on a given branch or on a single spur. Degree of symptom expression varied from year to year, but severely affected branches annually produced some mature fruits with external symptoms. No mechanisms have been proposed to explain the stunting of fruit on vigorous trees and the variations in degree of symptom expression by individual fruits.

In California, sweet cherries growing on mahaleb roots have been attacked by buckskin virus, which is related to western X-little cherry virus (17). Mature fruits were about normal in size, but the diseased trees often wilted and died within 2 years of infection (10). Schneider (12) found that the decline and death of diseased trees on mahaleb roots was caused by phloem necrosis near the graft union that girdled the trees, killed the roots, and caused the tops to wilt and die. In this instance he was able to demonstrate a definite locus and mode of virus action that could induce symptoms in the vegetative portions of the trees without producing characteristically variable effects upon the growth of the fruit.

A different situation was found in California in buckskin-diseased sweet cherry trees growing on mazzard roots. The infected trees remained alive for many years. Diagnostic symptoms were of 2 types: a) stunting of the mature fruits, and b) abnormal coloring of the leaves late in the growing season (10). Under these conditions Schneider (12) did not find consistent virus-induced changes in vegetative tissues that could cause the characteristic stunting of the fruits.

Although a direct relationship between growth of the embryo and growth of the enclosing peach or cherry has been known for some time (14), little has been published on the specific effects of viruses in the western X-disease group upon the seeds of the several hosts. Reeves et al (11) reported that seeds fail to develop and pits are discolored within the small peaches on western X-diseased trees. Stoddard et al (13) found that seeds do not develop within peaches and chokecherries showing symptoms of X-disease. Except in a preliminary report by Jones and Milbrath (7) and a report by Wilks and Milbrath (16), no mention can be found of the effects of western X-little cherry virus on seeds of diseased sweet cherries.

MATERIALS AND METHODS.—Samples for anatomical study were collected from healthy and diseased trees at the Mid-Columbia Branch Experiment Station, The Dalles, Oregon, and from nearby commercial orchards. The diseased trees were vigorous, and all evidence

→

Fig. 1-9. Collected at harvest time.—**1)** Healthy Lambert fruit.—**2)** Severely diseased Lambert fruit.—**3)** Healthy (2×); a) pit, b) fresh seed, c) air-dry seed.—**4)** Severely diseased (2×); a) pit, b) fresh seed, c) air-dry seed.—**5)** Parenchyma in mesocarp of healthy fruit (120×).—**6)** Parenchyma in mesocarp of severely diseased fruit (120×).—**7)** Functional sieve plate in phloem of fruit spur on which all fruit were severely diseased (1230×).—**8)** Rounded nucleus in parenchyma cell of the mesocarp of a healthy fruit (510×).—**9)** Spindle-shaped nucleus in parenchyma cell of the mesocarp of a severely diseased fruit (510×).

[1] Accepted for publication November 11, 1958.

Technical Paper No. 1167, Oregon Agricultural Experiment Station. The assistance of Dr. J. A. Milbrath in diagnosing and selecting trees for this study, and of Dr. J. M. Wilks in making oil determinations, is gratefully acknowledged.

[2] Associate Botanist.

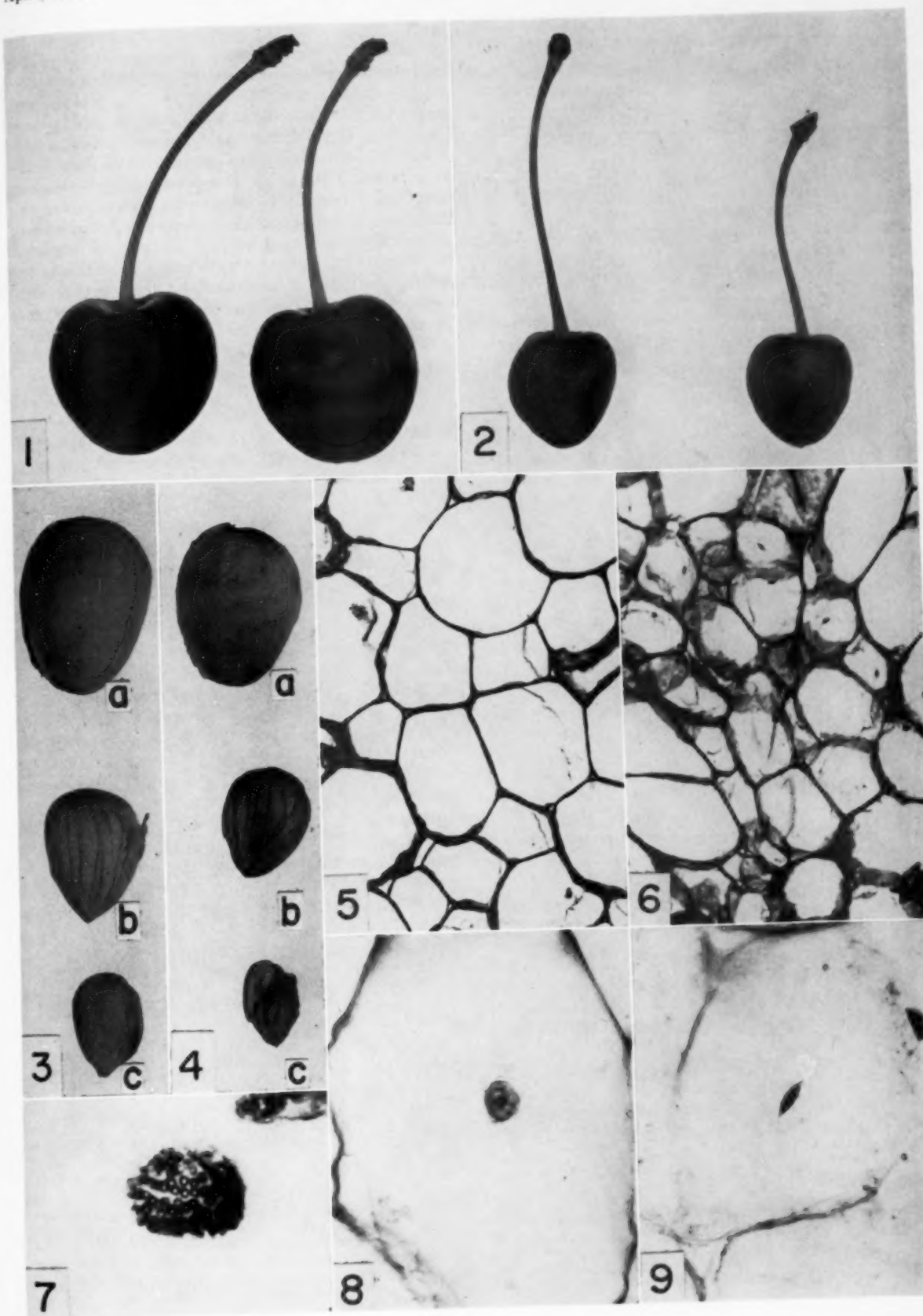

1
2
a
b
c
3
a
b
c
4
5
6
7
8
9

TABLE 1.—*Comparison of the number of parenchyma cells in the mesocarp of affected and unaffected sweet cherry fruits*[a]

Fruit type	Date collected	Mean number of cells per field of view	F value
Immature Lambert			
Healthy	May 20	19.2	
Diseased	May 20	20.2	0.91[b]
Mature Lambert			
Healthy	June 26	13.0	
Diseased	June 26	17.6	23.9**
Mature Bing			
Healthy	June 24	11.1	
Diseased	June 24	15.5	46.9**

[a] Cell size is inversely proportional to the number of cells per field of view.
[b] Difference between means is not significant.
** Difference between means is significant at the 1% level.

indicated that both healthy and diseased trees were growing on mazzard roots. The samples included developing and mature fruits, seeds, pedicels, leaves, petioles, and spurs. Samples from diseased trees were collected from 2 parts of each tree: a) the part producing fruit with severe symptoms, and b) the part producing normal-appearing fruit. Standard histological and histochemical procedures were used to prepare sections for study (2, 3, 6, 9). Most of the anatomical studies were made on samples from Lambert trees, but observations on Bing and Royal Ann (Napoleon) tissues were sufficient to show that they reacted to the virus as did the Lambert.

Seeds were collected at harvest time from 3 types of fruit: a) healthy, b) severely affected, and c) symptomless on diseased trees. The pits were cleaned, air-dried, cracked, and the dry seeds were removed and weighed. Average oil content was determined by grinding 100 air-dry seeds, extracting them for 32 hours in 100 ml of diethyl ether at room temperature, decanting two 25-ml aliquots, and allowing the solvent to evaporate at room temperature until the weight of each aliquot remained constant. The ether-soluble non-volatile residue was a yellow viscous liquid. It is reported as "oil" in Table 2, although it could contain other ether-soluble compounds.

The relative size of parenchyma cells in the mesocarp of healthy and diseased fruits was determined by counting the number of cells in a standard field of view while using an ocular reticule. No counts were made in areas adjacent to veins, because those areas contained many small cells not typical of the parenchyma that makes up the bulk of the mesocarp.

RESULTS AND INTERPRETATIONS.—*Anatomical studies.*—All organs and tissues from symptomless portions of diseased trees were basically the same as those from healthy trees of the same variety. Therefore, healthy tissues are compared only with tissues from the parts of diseased trees that produced fruit with severe symptoms.

Sections of spurs, leaves, petioles, and pedicels from severely diseased portions of trees were similar to those from healthy trees. There was no consistent evidence of necrotic phloem in any of the vegetative tissues. Sieve plates in the phloem from diseased trees appeared to be functional when stained with Lacmoid (3) (Fig. 7). Belkengren (1), using radioactive C^{14} in carbon dioxide, found that photosynthates were translocated from leaves into nearby fruits with severe symptoms. Two other observations also indicated that the characteristic stunting of fruits was not caused by a widespread degeneration of phloem in vegetative tissues: a) the normal growth and vigor of limbs and foliage on diseased trees, and b) the extreme variations in degree of symptom expression by fruits on a given portion of a diseased tree. All evidence pointed to changes localized within the fruit.

Consistent tissue differences between healthy and diseased fruits developed only during the preharvest flush of growth. Again, the differences were not due to abnormalities in the phloem. Most of the parenchyma cells in the mesocarp of mature healthy fruits were comparatively large and contained rounded nuclei, whereas those in severely diseased fruits were comparatively small and contained spindle-shaped nuclei (Fig. 5, 6, 8, 9; Table 1). The differences were not found in immature fruits collected about 5 weeks before harvest. Immature fruits from diseased and healthy trees had the same external appearance and internal anatomy. The young fruits on diseased trees were collected from marked spurs that had borne only severely diseased fruits during the previous year; at the following harvest too, only severely diseased fruits were found on these spurs. Under these conditions the assumption was made that the immature fruits collected from the marked spurs 5 weeks before harvest time would also have shown severe symptoms had they remained on the spurs until maturity. The above observations showed that the macro- and microscopic symptoms in mature fruits were induced by the virus during the 5 weeks preceding fruit ripening. Several workers have shown that the preharvest flush of growth in the mesocarp

TABLE 2.—*Average air-dry weight and "oil" content of healthy and diseased seeds from mature fruit*

Variety	Type of fruit used as seed source		
		Diseased tree	
	Healthy	Symptomless[a]	Severely affected
Air-dry weight per seed (g)			
Bing	0.05 (8)[b]	0.04 (6)	0.02 (7)
Royal Ann	0.05 (6)	0.05 (3)	0.02 (5)
Lambert	0.06 (5)	0.06 (3)	0.03 (5)
"Oil" content per seed[c] (g)			
Bing	0.017 (3)	0.011 (1)	0.002 (2)
Royal Ann	0.018 (3)	0.017 (1)	0.003 (2)
Lambert	0.025 (3)	0.019 (1)	0.003 (2)

[a] Normal-appearing fruit on a diseased tree.
[b] Numbers in parentheses indicate number of trees sampled.
[c] Ether-soluble non-volatile fraction.

of drupaceous fruits is due only to the enlargement of cells already present (4, 15).

Seed comparisons.—Fresh pits and seeds taken at harvest time from severely diseased fruits were about as large as those from healthy fruit of the same variety. When air-dried, all seeds from severely diseased fruits became shriveled, whereas most of those from healthy fruit remained plump (Compare Fig. 3 and 4). The relative plumpness of the air-dry seeds was indicated by average weights: a) healthy, 0.05 g; b) symptomless, 0.05 g; and c) severely diseased, 0.02 g (Table 2).

Sections of fresh seeds from severely diseased fruits at harvest showed that the radicle, hypocotyl, epicotyl, and cotyledons were comparable in gross structure to those from healthy fruits (Fig. 10, 11). The shriveling characteristic of air-dry seeds from severely diseased fruits could not be attributed to absence or degeneration of major organs in the seeds. The shriveling was due to a lack of reserve food in the diseased embryos. Chemical analyses have shown that cherry seeds contain oils (5). Histochemical tests made in this study showed that cherry embryos do not contain starch, but the cotyledons of healthy fresh embryos do contain many round droplets that were Sudan IV positive for oil; few or no oil droplets were found in similar sections from severely diseased seeds (Fig. 12, 13). The average amount of "oil" per mature seed was: a) healthy, 0.020 g; symptomless, 0.016 g; and c) severely diseased, 0.003 g (Table 2).

Three hypotheses can be offered: a) the virus inhibited translocations of foods, b) foods moved in, but were respired or moved out before they could be converted into oil, or c) the virus directly inhibited the conversion of soluble foods into oil by the cells of the embryo. The first hypothesis appears untenable, because the phloem is intact and photosynthates do move into the fruits (1). Testing the other hypotheses will require more physiological evidence than is now available.

Discussion.—All evidence on virus action indicated that the locus was within each fruit and seed, the

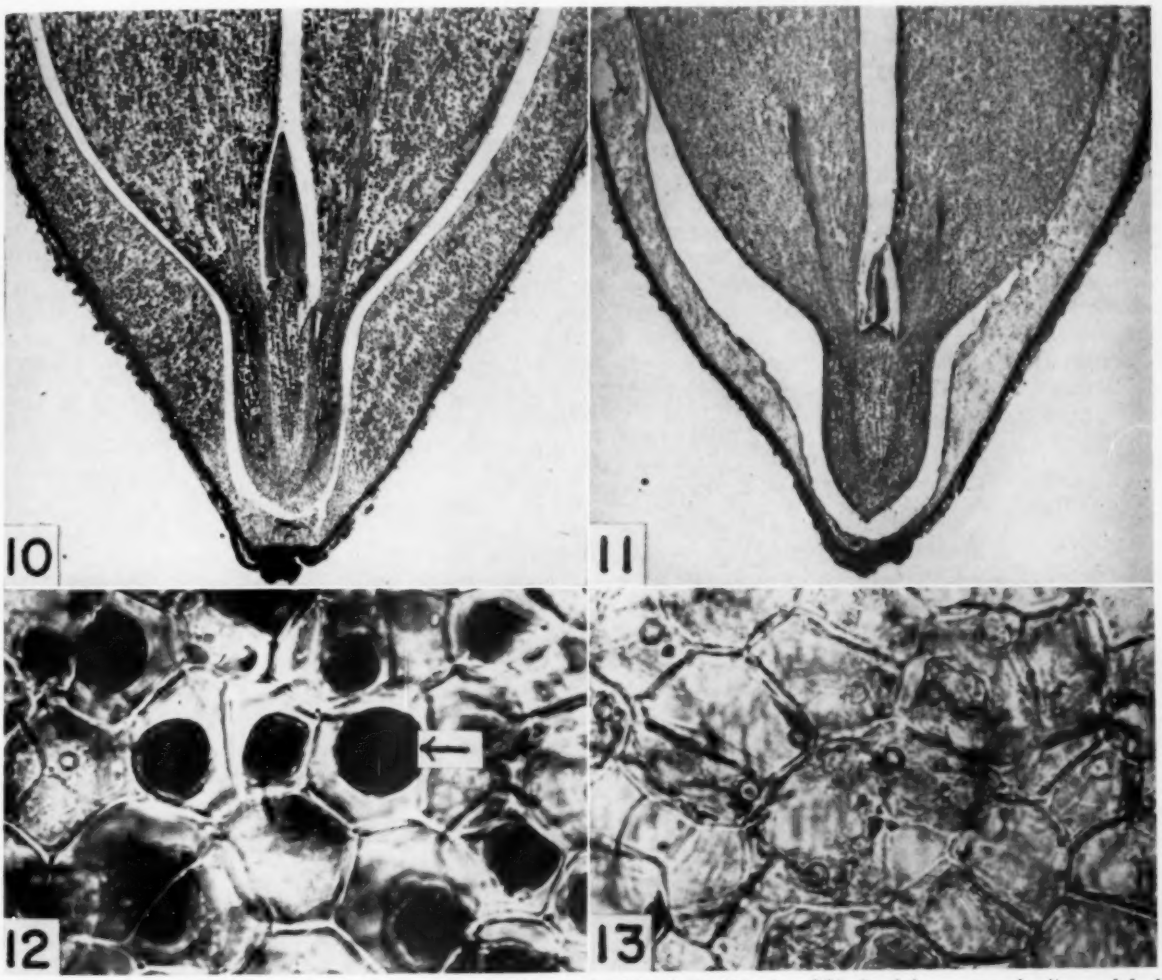


Fig. 10-13. Collected at harvest time.—**10)** Seed from healthy fruit (20×).—**11)** Seed from severely diseased fruit (20×).—**12)** Fresh cotyledon from healthy fruit; stained n Sudan IV (510×); large fat droplet at arrow.—**13)** Fresh cotyledon from severely diseased fruit; stained in Sudan V (510×).

time was during the preharvest flush of growth, and the mode was inhibition of cell enlargement in the mesocarp and prevention of oil accumulation within each developing seed. The virus caused the changes within individual fruits and seeds without inducing diagnostic anatomical symptoms within the vegetative portions of diseased limbs that retained their vigor. Because the changes were restricted to individual fruits and seeds, the degree of symptom expression varied from one fruit to another. This variation is characteristic of western X-little cherry disease in sweet cherries in Oregon.

Changes induced by viruses in the western X-disease group are not restricted to those described above. Symptoms vary with the circumstances (11), and these variations indicate that several mechanisms leading to virus-induced changes are in operation. Three types of changes now are known. Schneider (12) found that necrotic phloem is responsible for the death of diseased sweet cherry trees on mahaleb roots in California. Nichols (8), in Idaho, found that the virus inhibits cell division in diseased fruits prior to, during, or shortly after flowering. He also presented data (8, Table XVIII, pg. 42) indicating that the virus inhibits enlargement of cells in the mesocarp of diseased fruits during the preharvest flush of growth. In Oregon, diagnostic changes in vigorous trees are limited to the fruits and seeds during the preharvest flush of growth. Different combinations of the three types of virus action could be responsible for the wide range of symptoms reported for western X-disease in the United States. Each situation must be investigated to determine which combination of loci, times, and modes of virus-action is in operation.

Department of Botany and Plant Pathology
Oregon State College
Corvallis, Oregon

LITERATURE CITED

1. Belkengren, R. O. Unpublished data. Oregon State College, Corvallis, Oregon.
2. Esau, Katherine. 1941. Phloem anatomy of tobacco affected with curly top and mosaic. Hilgardia **13:** 437-490.
3. Esau, Katherine. 1948. Phloem structure in the grapevine, and its seasonal changes. Hilgardia **18:** 217-296.
4. Esau, Katherine. 1953. Plant anatomy. Wiley and Sons, Inc., New York. 735 p.
5. Jamieson, G. S. 1943. Vegetable fats and oils. Ed. 2. Reinhold Publishing Corp., New York. 508 p.
6. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., New York. 523 p.
7. Jones, L. E., and J. A. Milbrath. 1952. The influence of western X-little cherry virus upon seed size and viability. (Abstr.) Phytopathology **42:** 515.
8. Nichols, C. W. 1949. Investigations of the little cherry virus disease in Idaho. Unpublished M.S. thesis. University of Idaho, Moscow.
9. Rawlins, T. E., and W. N. Takahashi. 1952. Technics of plant histochemistry and virology. The National Press, Millbrae, Calif. 125 p.
10. Rawlins, T. E., and H. E. Thomas. 1951. Buckskin. *In* Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr., Agr. Handbook 10, p. 98-102.
11. Reeves, E. L., E. C. Blodgett, T. B. Lott, J. A. Milbrath, B. L. Richards, and S. M. Zeller. 1951. Western X-disease. *In* Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr., Agr. Handbook 10, p. 43-52.
12. Schneider, H. 1945. Anatomy of buckskin-diseased peach and cherry. Phytopathology **35:** 610-635.
13. Stoddard, E. M., E. M. Hildebrand, D. H. Palmiter, and K. G. Parker. 1951. X-disease. *In* Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr., Agr. Handbook 10, p. 37-42.
14. Tukey, H. B. 1936. Development of cherry and peach fruits as affected by destruction of the embryo. Botan. Gaz. **98:** 1-24.
15. Tukey, H. B., and J. O. Young. 1939. Histological study of the developing fruit of sour cherry. Botan. Gaz. **100:** 723-749.
16. Wilks, J. M., and J. A. Milbrath. 1956. Comparative studies of the virus diseases western X little cherry and little cherry. Phytopathology **46:** 596-599.
17. Zeller, S. M., and J. A. Milbrath. 1950. The recovery of western X-disease of peach from Montmorency cherry and its relation to Buckskin of sweet cherry. Phytopathology **40:** 707-711.

THE EFFECT ON VARIOUS CULTURES OF ERYSIPHE GRAMINIS F. SP. HORDEI OF THE GENES IN BARLEY THAT CONDITION RESISTANCE TO CULTURE CR3[1]

J. G. Moseman and C. W. Schaller[2]

SUMMARY

The F_3 families from crosses of 7 varieties of barley, *Hordeum vulgare*, each with a single gene conditioning resistance to culture CR3 of *Erysiphe graminis* f. sp. *hordei*, were studied. It was determined that the gene conditioning resistance to culture CR3 also conditioned resistance to certain cultures of other races of *Erysiphe graminis* f. sp. *hordei*, as follows: gene $M1_a2$ in Black Russian to A8, 28A1, A9, R11, and CAN12; $M1_a3$ in Ricardo to A8, 28A1, A9, R11, and CAN12; $M1_p$ in Psaknon to A8, 28A1, A9, R11, and CAN12; $M1_g$ in Goldfoil to A8, 28A1, and A9; $M1_a$? in Durani to 28A1, A9, R11, and CAN12; $M1_h$ in Hanna to A8; and $M1_k$ in Kwan to 28A1 and A9.

INTRODUCTION.—In recent years the genes in barley that condition resistance to *Erysiphe graminis* DC. ex Merat. f. sp. *hordei* Em. Marchal and the physiologic races of that pathogen have been studied by several workers. Many new genes conditioning such resistance have been identified and their relationship determined, and several new physiologic races of the pathogen have been isolated. It would be of great value to both plant breeders and plant pathologists if they knew which races each gene conditioned resistance to.

Barley varieties with resistance to culture CR3 of race 3 of *E. graminis* f. sp. *hordei* from California, conditioned by single genes, are known to be resistant to cultures of other races. This study was undertaken to determine whether these same genes condition the resistance to other cultures.

REVIEW OF LITERATURE.—The literature pertaining to genes that condition resistance to specific races of *E. graminis* f. sp. *hordei* has been reviewed by Smith (13) and Moseman and Starling (8). Only information not discussed in those papers is mentioned herein.

Nover and Mansfeld (10, 11) reported that only 3 of 702 varieties of spring barley were immune to 4 races of powdery mildew in Germany, and 6 of 225 varieties of winter barley were resistant or immune to 7 races of the pathogen.

From 15,000 seeds subjected to X rays, Hänsel and Zakovsky (2, 3), in Austria, obtained 72 plants that were more resistant to powdery mildew than untreated plants of the parents. One of these mutants, which they designated 3502/53, was resistant to 5 races of the pathogen.

Hiura and Heta (4), in Japan, found 12 varieties that were immune to 11 races of powdery mildew that they identified. In a later study, Hiura, Heta, and Akabori (5) reported that some of these varieties had 1 gene conditioning resistance to all 11 races, but that other varieties had 1 gene conditioning resistance to some races and 2 or 3 genes conditioning resistance to other races.

MATERIAL AND METHODS.—Seed of the 7 resistant barley varieties Goldfoil, Hanna, Kwan, Psaknon, Black Russian, Ricardo, and Durani, each with 1 gene conditioning resistance to culture CR3 of powdery mildew, was from the source used in original studies in which the genes were identified. In Table 1 the varieties are designated by C.I. number[3] and gene that conditions resistance to culture CR3. The relationships have been determined for all the genes (7, 12) except that in Durani, which is at or near the $M1_a$ locus.[4]

Varieties Atlas (C.I. 4118) and Manchuria (C.I. 2330) were used as susceptible parents. Atlas was used in the studies in which the individual genes conditioning resistance to culture CR3 were identified. It was susceptible to culture CR3, and moderately resistant to some of the other cultures, whereas Manchuria was susceptible to all cultures.

Each of the 6 cultures of powdery mildew—CR3, A8, 28A1, A9, R11, and CAN12—used in this study originated from either a single conidium or a single ascospore. Table 2 lists the physiologic races indicated by the reaction of the 6 differentiating varieties, as described by Newton and Cherewick (9), and the locations where the original material was collected.

Plants were inoculated in the first-leaf stage, and readings were made 8–10 days later. Inoculations were made in a growing room at 66–69°F with 550 ft-c of light from cool, white, fluorescent tubes for 16 hours a day. Reactions of plants to infection were recorded by a method similar to that published by Moseman (6). The general description of reaction types is as follows: 0 = immune; 1 = highly resistant; 2 = moderately resistant; 3 = moderately susceptible; and 4 = very susceptible.

The reactions of the F_3 families to inoculation with culture CR3 were compared with their reactions to the other cultures. Twenty-five plants were used to determine the reaction of each F_3 family to inoculation with culture CR3. Fifteen plants of F_3 families susceptible, and 25 plants of F_3 families segregating or resistant, to culture CR3 were then inoculated with the other cultures. Since there was insufficient seed

[1] Accepted for publication November 11, 1958.
Cooperative investigations of the U. S. D. A., ARS, Crops Research Division, and the California Agricultural Experiment Station, Davis.

[2] Respectively, Plant Pathologist, Crops Research Division, ARS, U. S. D. A., Beltsville, Maryland, and Assoc. Prof. Agronomy, Univ. of California, Davis.

[3] C.I. number refers to the accession number of the Cereal Crops Research Branch, Crops Research Division.

[4] Unpublished data.

TABLE 1.—*Genes in 7 barley varieties conditioning resistance to culture CR3 of Erysiphe graminis hordei*

Variety	C.I. no.	Genes conditioning resistance[a]	Variety	C.I. no.	Genes conditioning resistance
Goldfoil	928	Ml_g	Black Russian	2202	Ml_a2
Hanna	906	Ml_h	Ricardo	6306	Ml_a3
Kwan	1016	Ml_k	Durani	6316	Ml_a?
Psaknon	6305	Ml_p			

[a] Genes conditioning resistance as reported by Schaller and Briggs (12) and Moseman and Schaller (7). Information on the gene that conditions resistance in Durani is from upublished data.

of some F_3 families to test with culture CR3 and all the other cultures, other F_3 families were used for inoculation with the other cultures. Therefore, reactions to inoculation with culture CR3 could be compared to reactions with other cultures, but comparisons could not be made among the reactions with other cultures.

F_3 families from a cross were not tested with a culture to which both parents were susceptible. Since the parents were susceptible, F_3 families from the crosses with Goldfoil and Kwan were not tested with culture CAN12, and those from the cross with Hanna were not tested with cultures 28A1, A9, R11, and CAN12.

RESULTS.—A preliminary study was made with F_3 families from the 4 crosses of Atlas with Black Russian, Ricardo, Psaknon, and Durani. F_3 families from these crosses, homozygous susceptible or resistant to culture CR3, were inoculated with other cultures. The F_3 families resistant to culture CR3 were likewise resistant to the other cultures. This finding indicates that, in each variety, the same gene, or closely linked genes, condition resistance to all these cultures, but it does not eliminate the possibility that the varieties may have additional genes conditioning resistance. F_3 families susceptible to culture CR3 were likewise susceptible to culture R11, but in their reaction to the other cultures were either homozygous moderately resistant, segregating for moderate resistance and susceptibility, or homozygous susceptible.

TABLE 2.—*Description and origin of the 6 cultures of Erysiphe graminis hordei*

Stock culture no.	Physiologic race[a]	Originated from a single	From barley plants grown at
CR3	3	Conidium	Davis, California
A8	6	Ascospore	Statesville, North Carolina
28A1	8	Ascospore	Ithaca, New York
A9	9	Ascospore	Statesville, North Carolina
R11	11	Conidium	Blacksburg, Virginia
CAN12	12	Ascospore	Winnipeg, Manitoba

[a] The physiologic race of each culture was determined by the reaction of the 6 differential varieties: Black Hulless, C.I. 666; Chevron, C.I. 1111; Goldfoil, C.I. 928; Heil's Hanna, C.I. 682; Nepal, C.I. 595; and Peruvian, C.I. 935, as described by Newton and Cherewick (9).

These different reactions to cultures A8, 28A1, A9, and CAN12 could be explained by the fact that Atlas was moderately resistant to the latter group.

Since Atlas was not susceptible to all cultures, crosses were made with Manchuria, which was susceptible to all cultures. Table 3 shows the reaction to inoculation with culture CR3 of F_3 families from crosses of 7 varieties with Manchuria. Previous tests (7, 12) of progeny from crosses of these varieties with Atlas have shown that each variety has 1 gene conditioning resistance to culture CR3. Data in Table 3 likewise indicate that each variety, when crossed with Manchuria, has 1 gene conditioning resistance to culture CR3.

F_3 families from the crosses with Manchuria also were inoculated with other cultures of powdery mildew. Table 4 shows the number of F_3 families from each of 7 crosses with Manchuria that were inoculated with culture CR3 and other cultures. All the F_3 families from these crosses showed the same reactions to inoculation with culture CR3 and the other cultures tested. The plants in families homozygous resistant or susceptible, respectively, reacted the same as the resistant or susceptible parent. Plants in families segregating for resistance and susceptibility had an intermediate reaction only in crosses in which the gene conditioning resistance was not completely dominant.

DISCUSSION.—Reaction to infection can be visualized as the interaction of 2 independent biological systems, one in the host and the other in the pathogen. Flor (1) has shown for flax rust, *Melampsora lini* (Ehrenb.) Lev., and flax, *Linum usitatissimum* L., that there are complementary genes for pathogenicity in the pathogen and for conditioning resistance in the host. If this relationship is true for the pathogen *Erysiphe graminis hordei* and its host, *Hordeum vulgare*, then it could be expected that the gene in a variety conditioning resistance to culture CR3 could likewise condition resistance to other cultures provided each of the cultures lacked the complementary gene for virulence to the 1 gene conditioning resistance. Other genes for pathogenicity in the pathogen or for resistance in the host would not be involved in this interaction.

In this study, 7 distinct genes conditioned resistance to culture CR3. If Flor's hypothesis is valid for these

TABLE 3.—*Seedling reactions of F_3 families from crosses of 7 varieties of barley with Manchuria to inoculation with culture CR3 of Erysiphe graminis hordei*

Variety crossed on Manchuria	Number of F_3 families			Ratio	Value of P for ratio indicated
	Resistant	Segregating	Susceptible		
Goldfoil	9	25	10	1:2:1	0.50
Hanna	10	18	11	1:2:1	0.80
Kwan	11	19	9	1:2:1	0.80
Psaknon	12	26	12	1:2:1	0.20
Black Russian	14	34	9	1:2:1	0.20
Ricardo	6	24	8	1:2:1	0.20
Durani	5	18	7	1:2:1	0.30

TABLE 4.—*Number of F_3 families from crosses of 7 varieties of barley, each with Manchuria, that were inoculated with culture CR3 and other cultures of Erysiphe graminis hordei nonvirulent to the resistant parent and seedling reactions*

	Reaction of F_3 families to culture CR3 and other cultures[a]	Number of F_3 families inoculated with culture CR3 and cultures[b]				
		A8	28A1	A9	R11	CAN12
Black Russian × Manchuria	R	5	5	5	5	10
	Seg	5	4	5	5	–
	S	5	5	5	5	5
	Total	15	14	15	15	15
Ricardo × Manchuria	R	4	4	4	4	4
	Seg	6	6	6	6	6
	S	5	5	5	5	5
	Total	15	15	15	15	15
Durani × Manchuria	R	*	5	5	5	5
	Seg	*	5	5	10	5
	S	*	5	5	5	5
	Total	*	15	15	20	15
Psaknon × Manchuria	R	5	5	5	5	8
	Seg	5	5	5	5	–
	S	5	5	5	5	7
	Total	15	15	15	15	15
Goldfoil × Manchuria	R	5	5	7	*	–
	Seg	5	4	8	*	–
	S	5	4	5	*	–
	Total	15	13	20	*	–
Hanna × Manchuria	R	4	–	–	–	–
	Seg	6	–	–	–	–
	S	5	–	–	–	–
	Total	15	–	–	–	–
Kwan × Manchuria	R	*	5	5	*	–
	Seg	*	5	5	*	–
	S	*	5	5	*	–
	Total	*	15	15	*	–

[a] All F_3 families reacted the same to inoculation with culture CR3 and to the other cultures.

[b] Some crosses were not tested with all the cultures, because (–) both parents were susceptible or (*) there was insufficient seed of individual F_3 families.

organisms also, then culture CR3 must have 7 genes for avirulence, 1 complementary to each gene conditioning resistance to it. The other cultures must possess the same genes for avirulence complementary to the genes conditioning resistance on which they were avirulent as does culture CR3, since the same genes were found to condition resistance in the host to culture CR3 and the other cultures. Information was not obtained regarding genes conditioning resistance in the host for which all cultures possessed the complementary gene for virulence, or on genes for pathogenicity for which none of the varieties had the complementary gene conditioning resistance.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND
AND
DEPARTMENT OF AGRONOMY
UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA

LITERATURE CITED

1. FLOR, H. H. 1955. Host-parasite interaction in flax rust—its genetics and other implications. Phytopathology **45**: 680-685.
2. HÄNSEL, H., AND J. ZAKOVSKY. 1956. Röntgeninduzierte Mutanten der Vollkorngerste (Hordeum distichum nutans). I. Bestrahlung und Auslese auf Mehltauresistenz. Bodenkultur **9**: 50-64.
3. HÄNSEL, H., AND J. ZAKOVSKY. 1956. Mildew-resistant barley mutants induced by X-rays. Euphytica **5**: 347-352.
4. HIURA, U., AND H. HETA. 1955. Studies on the disease-resistance in barley. III. Further studies on the physiologic races of Erysiphe graminis hordei in Japan. Ber. Ohara Inst. landwirtsch. Biol. Okayama Univ. **10**(3): 135-152.
5. HIURA, U., H. HETA, AND T. AKABORI. 1956. Studies on the disease resistance in barley. VIII. Factors for resistance to mildew. (3). Nôgaku Kenkyû **44**(1): 11-33.
6. MOSEMAN, J. G. 1956. Physiological races of Erysiphe graminis f. sp. hordei in North America. Phytopathology **46**: 318-322.
7. MOSEMAN, J. G., AND C. W. SCHALLER. 1958. Separation of genes conditioning resistance to Erysiphe graminis f. sp. hordei by two pathogenically different cultures. (Abstr.) Phytopathology **48**: 463.
8. MOSEMAN, J. G., AND T. M. STARLING. 1958. Genetics of resistance of the barley varieties Ricardo and Modia to several cultures of Erysiphe graminis f. sp. hordei. Phytopathology **48**: 601-604.
9. NEWTON, MARGARET, AND W. J. CHEREWICK. 1947. Erysiphe graminis in Canada. Can. J. Research, C, **25**: 73-93.
10. NOVER, I., AND R. MANSFELD. 1955. Resistenzeigenschaften im Gersten- und Weizensortiment Gatersleben. I. Prüfung von Sommergersten auf ihr Verhalten gegen Erysiphe graminis DC. f. sp. hordei Marchal. Die Kulturpflanze **3**: 105-113.
11. NOVER, I., AND R. MANSFELD. 1956. Resistenzeigenschaften im Gersten- und Weizensortiment Gatersleben. II. Prüfung von Wintergersten auf ihr Verhalten gegen Erysiphe graminis DC. f. sp. hordei Marchal. Die Kulturpflanze **4**: 341-349.
12. SCHALLER, C. W., AND F. N. BRIGGS. 1955. Inheritance of resistance to mildew, Erysiphe graminis hordei, in the barley variety, Black Russian. Genetics **40**: 421-428.
13. SMITH, L. 1951. Cytology and genetics of barley. Botan. Rev. **17**(3): 133-202.

VARIATION IN PHYTOPHTHORA PALMIVORA ISOLATED FROM CACAO AND RUBBER[1]

R. G. Orellana[2]

SUMMARY

Isolates of *Phytophthora palmivora* from *Theobroma cacao* and *Hevea brasiliensis*, when grown separately on various media, differed in appearance, rate and manner of growth, amount of sporulation, and spore size. It was not possible to induce successful mating in Costa Rica between local isolates from cacao and rubber, or in Ceylon among 4 isolates from cacao (2 from Ceylon and 1 each from Costa Rica and Ghana) and 3 isolates from rubber (1 each from Costa Rica and Ceylon and *P. heveae* from Malaya). Disease symptoms were striking only when cacao pods and seedlings were inoculated with *P. palmivora* from cacao and rubber fruits, and seedlings were inoculated with isolates from rubber. Because of morphological, physiological, and specific pathogenic differences of *P. palmivora* isolated from cacao and rubber, it is proposed that the species described by Coleman (2) and by Thompson (18) be transferred to varietal status under *P. palmivora* Butl. as, respectively, *P. palmivora* Butl. var. *theobromae* (Colem.) comb. nov. (basionym: *P. theobromae* Colem.); and *P. palmivora* Butl. var. *heveae* (Thomps.) comb. nov. (basionym: *P. heveae* Thomps.).

Cacao (*Theobroma cacao* L.) and rubber (*Hevea brasiliensis* Muell.-Arg.) grow under similar conditions in the moist tropics of the world. In certain regions they are interplanted. One of the most serious and widespread diseases of these trees is due to *Phytophthora palmivora* Butl., which induces on cacao the disease known as black pod rot, and on rubber the black stripe of the tapping panel. On cacao, this fungus also causes chupon wilt, leaf blight, and trunk canker, particularly on cacao containing the Criollo strain. On rubber, *P. palmivora* also causes rotting of the fruit and defoliation. The results of an investigation of variation of isolates of *P. palmivora* from cacao and rubber are reported herein (14).

TAXONOMY OF THE ISOLATES FROM CACAO AND RUBBER.—Diseases now attributed to *P. palmivora* were formerly ascribed to various other fungi. Tucker (19) listed the synonyms of *P. palmivora* and the host range, and concluded that biological specialization exists in this fungus. Additional studies on the host range and inoculation tests were reported by Hartley (8), Reinking (15), Demandt (5), Rutgers (16), Stoughton-Harris (17), Gadd (6), and Dade (3). Evidence of heterothallism was reported by Ashby (1), Gadd (7), Leonian (10), and Narasimhan (12). Dastur (4), Thompson (18), Leonian (11), and Ashby (1), among others, have noted differences in isolates of *P. palmivora* from rubber and cacao.

MATERIALS AND METHODS.—Pure cultures of *P. palmivora* were obtained from infected Trinitario cacao pods and Hevea fruits. For the study of the growth of these isolates, cacao-dextrose broth was prepared in series containing 50, 100, 150, 200, 250, and 300 g of cacao shell tissue plus 5 g of dextrose per liter. The dry weight of the mycelium in the broth was recorded after 15 days.

The ability of the cacao and rubber isolates to produce oospores was tested by growing them in PDA (potato-dextrose agar), in 3% tomato-juice agar, and in PDA containing 3 concentrations of malachite green hydrochloride (1.0, 0.1, and 0.01 ppm). Sporangia were measured from cultures grown on PDA.

Young seedlings in nurseries and in the greenhouse, woody stems on living trees in the field, and detached stems and fully developed but unripe fruits of cacao and rubber in the laboratory were used for pathogenicity tests and were cross-inoculated with the 2 types of *Phytophthora* isolates. These tissues were inoculated without wounding. Inocula were prepared by scraping spores from cultures grown in non-acidulated PDA medium and suspending them in sterile water.

The Costa Rican investigations included isolates obtained locally from cacao and from rubber trees. The work in Ceylon included the Costa Rican isolates among others,[2] as follows: (A) *P. heveae*, 36528 from Malaya; (B) *P. palmivora*, from Ceylon (from rubber); (C) *P. palmivora*, from Costa Rica (from rubber); (D) *P. palmivora*, from Costa Rica (from cacao); (E) *P. palmivora*, 63552 from Ghana (from cacao); (F) *P. palmivora*, from Vykumbra, Ceylon (from cacao); and (G) *P. palmivora*, from Kundasale, Ceylon (from cacao). *P. theobromae* was not available.

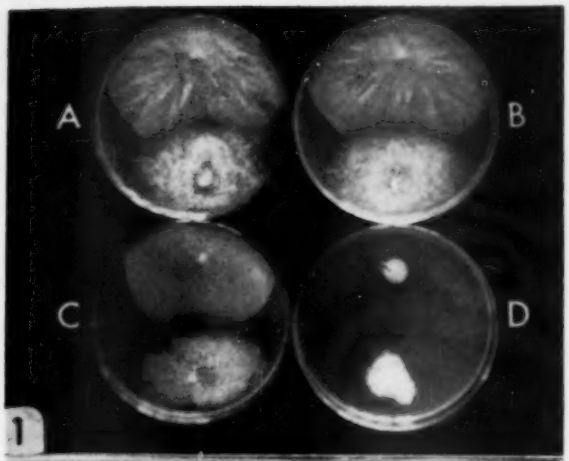
EXPERIMENTAL RESULTS.—*Morphology of the isolates.*—From the beginning the developing colonies of the 2 types of isolates showed striking differences in PDA, with or without malachite green hydrochloride (Fig. 1), or in cacao-dextrose agar. The rubber isolates grew in multi-pointed concentric waves, were

[1] Accepted for publication November 11, 1958.

[2] Present address: Oilseed and Industrial Crops Research Branch, U. S. D. A., University of Florida, Gainesville, Florida.

Investigation begun while author was on the staff of the Inter-American Institute of Agricultural Sciences, Costa Rica, and continued during FAO assignment in Ceylon.

The author is indebted to W. T. Lellis, Agricultural Experiment Station, Uruçuca, Bahia, Brazil, for assistance in the first phase of this work; to L. A. Gunasekera and M. B. Dissanayake, Plant Pathology Department, Ministry of Agriculture, Peradeniya, Ceylon, for assistance in its final portion; to H. D. Dade, Commonwealth Mycological Institute, England, for supplying isolates 36528 and 63552; and to A. Riggenbach, Rubber Institute, Ceylon, for supplying isolate of *P. palmivora* from rubber in Ceylon.



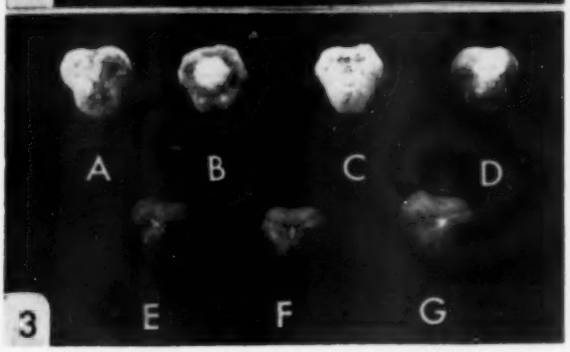


FIG. 1–3.—**Fig. 1.** Growth of *P. palmivora* on potato-dextrose agar containing malachite green hydrochloride at the following concentrations: A) zero, B) 0.01 ppm, C) 0.1 ppm, and D) 1.0 ppm. The rubber isolates are in the upper part of the plates and the cacao isolates in the lower. **Fig. 2.** Cacao pods inoculated with rubber isolates (A,B,C) and cacao isolates (D,E,F) of *P. palmivora*. **Fig. 3.** Rubber fruits inoculated with rubber isolates (A,B,C) and cacao isolates (D,E,F,G) of *P. palmivora*.

hyaline in appearance, and were somewhat appressed, with abundant mycelium submerged in the media. In 3% tomato-juice agar, only the rubber isolates produced aerial mycelial growth. The cacao isolates in all media formed hyaline and slightly raised colonies, with abundant mycelial growth and sporulation.

Growth differences.—Fig. 4 shows the total dry-weight curve of the Costa Rican cacao and rubber isolates in the series of media containing increasing amounts of cacao shell tissue. Weight of the isolates was maximum at the highest concentration of shell used. The growth of the rubber isolates, however, was less, and less influenced by cacao tissue content. When the 2 growth rates were compared, that of the cacao isolates was always higher than that of the rubber isolates.

According to Leonian (9), malachite green hydrochloride induces an excellent differential growth, particularly in Phytophthora species. When the Costa Rican isolates of Phytophthora from cacao and from rubber were grown in PDA plus malachite green (Fig. 1), a differential tolerance to this compound was demonstrated. The rubber isolates grew faster than the cacao isolates at 0.01 and 0.1 ppm of malachite green, but did not grow at 1.0 ppm, the highest concentration used. The cacao isolates were not as sensitive to this compound as were those from rubber; they were able to grow well at the lowest 2 concentrations of malachite green, and to some extent at 1.0 ppm. Sporulation of the 2 strains, either of sexual or asexual spores, failed to occur in these substrata. In conclusion, therefore, malachite green hydrochloride can be used as an aid to demonstrate the specificity of the 2 strains of Phytophthora by differences in their ability to utilize it.

With regard to temperature requirements of these 2 types of isolates, it was learned that they grew and sporulated abundantly at 27°C±2, the temperature of the laboratory. However, when these isolates were grown at a constant temperature of 37°C, neither isolate increased in weight significantly. This temperature can therefore be considered the maximum thermal level for their growth. On the other hand, there was a differential growth response when the

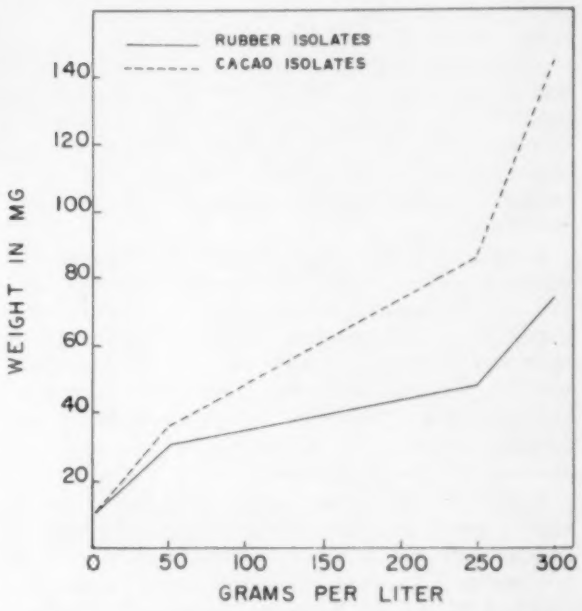


Fig. 4. Average dry weight at the end of 15 days of the mycelium of Costa Rican cacao and rubber isolates of *P. palmivora* grown on media containing 50, 100, 150, 200, 250, and 300 g of cacao shell tissue plus 5 g of dextrose per liter.

cacao and the rubber isolates were grown at 13°C±2. The cacao isolates grew more abundantly than the rubber isolates in terms of dry weight of the mycelium. Also, since the average dry weight of these isolates at this temperature was quite small, it is possible to state that both were approaching their minimum thermal level for growth.

Sporulation and sporangium size.—In cacao-dextrose agar the cacao isolates developed normal and abundant sporangia and chlamydospores. They also produced abnormal nodulations (in clusters and terminally and laterally on hyphal tips) similar to the vesicles observed in *P. cinnamomi* by Tucker (19). These nodules were more profuse when the amount of cacao shell tissue was increased in the media. In cacao-dextrose agar the rubber isolates produced only a few spores, and nodulations seldom occurred. In both solid and liquid media, sporulation of the isolates from rubber was generally scant, and slower than that of the isolates from cacao. Typical oospores never occurred in either cacao or rubber isolates. Throughout this entire investigation the only observation of what appeared to be oospores was made in 1953, by Orellana (13), within the uppermost cavities of the mesocarp in detached cacao pods inoculated with Costa Rican strain 21 of *P. palmivora* isolated from cacao.

Several exploratory measurements were made of sporangia from the 2 types of isolates grown on PDA. A final measurement was made of 100 sporangia of each type. From this it was found that, in Costa Rica, the mean length for the cacao sporangia was 38.22μ, and that for the rubber sporangia was 30.70μ. In width, too, the trend of differences in size was the same: 30.99μ for the cacao sporangia, and 18.25μ for those from rubber. Statistical analysis indicated that the mean lengths of the 2 types of isolates differed significantly. The mean widths of the 2 types likewise differed significantly. These same differences in sporangial dimensions were found when cacao and rubber isolates from other regions of the world were compared in Ceylon.

Sexuality of the cacao and rubber isolates.—Attempts to induce successful mating between the cacao and rubber isolates failed when the isolates were grown at room temperature in PDA, in tomato-juice agar, and at the 3 concentrations of malachite green in PDA. In artificial media, except PDA containing malachite green, sporangia and chlamydospores were formed profusely by the cacao isolates, and only sparsely by the rubber isolates.

A further attempt was made in Ceylon to induce mating among reciprocal combinations of the 3 rubber isolates with the 4 cacao isolates in PDA at room temperature. Although no typical sexual spores were observed throughout this experiment, a dark zone of growth became evident at the junction when the 2 colonies, one of which was the rubber isolate from Ceylon, approached. In other combinations, the partner colonies either exhibited a zone of inhibited growth, suggesting a mutual antagonism, or, in only a few combinations, they overlapped.

It is concluded that the cacao and the rubber isolates of *P. palmivora* are incompatible, and that there is no possibility of hybridization, a fact that indicates the strong individuality of the cacao and rubber strains.

Pathogenicity of the isolates.—Spore suspensions of *P. palmivora* from cacao inoculated on unwounded leaf petioles and terminal buds of young rubber seedlings in the greenhouse and on detached stems of adult trees in the laboratory caused blackening and defoliation in about 7–8 days, but the reaction was less severe than when the spores were placed on comparable parts of the cacao plants. On woody stems of rubber trees in the field, moderate cankering was induced by wound inoculation with spore suspensions of the cacao isolates, but expression of this symptom was delayed 3 months.

Spore suspensions of *P. palmivora* from rubber were inoculated on comparable parts of cacao plants in the laboratory, greenhouse, nursery, and field. No infection resulted.

Young seedlings of cacao inoculated on their stems with isolates from cacao, and young seedlings of rubber inoculated on their stems with isolates from rubber developed a severe blackening on the stems within a few days under greenhouse conditions. These seedlings later collapsed. Inoculations of rubber seedlings with the cacao isolates, and vice versa, left the plants intact.

Fully developed, unwounded, detached cacao pods inoculated with rubber isolates remained uninfected (Fig. 2). However, incipient rotting did develop in some rubber fruits inoculated with spores of *P. palmivora* from cacao (Fig. 3).

Cross-inoculation experiments, repeated in Ceylon, were expanded to include the 4 cacao isolates and 3 rubber isolates from various cacao- and rubber-growing regions of the world. When the cacao isolates from Ceylon, Costa Rica, and Ghana were tested for pathogenicity on cacao pods, the Costa Rican isolate was the most pathogenic, in spite of the fact that it had been in culture for over a year (Fig. 2). The Ceylonese cacao isolate was less pathogenic than the cacao isolate from Ghana. Also, some isolates of the cacao strain seem to be more adaptable to rubber, as shown when the cacao isolate from Costa Rica infected rubber fruits (Fig. 3). These differences in pathogenicity indicate that there is more latitude for pathogenic variation among cacao isolates than among rubber isolates.

Discussion.—These results demonstrate that the 2 types of isolates possess different pathogenic capability and differ in morphological and physiological characteristics. This behavior, substantiated by studying isolates from the main cacao- and rubber-producing regions of the world, justifies the transfer to varietal status under *P. palmivora: P. palmivora* Butl. var. *theobromae* (Colem.) comb. nov. (basionym: *P.*

theobromae Colem. Mysore Agr. Dept. Mycol. Ser. Bull. 2, 92 p. 1910) for the strain that attacks *T. cacao;* and *P. palmivora* Butl. var. *heveae* (Thomps.) comb. nov. (basionym: *P. heveae* Thomps. Malayan Agr. J. 17: 53-100. 1929) for the strain that attacks *H. brasiliensis.*

Department of Plant Pathology
Ministry of Agriculture
Peradeniya, Ceylon

LITERATURE CITED

1. Ashby, S. F. 1929. Strains and taxonomy of Phytophthora palmivora Butl. (P. faberi Maubl.). Brit. Mycol. Soc. Trans. **14**: 18-33.
2. Coleman, L. 1910. Diseases of the Areca palm. I. Koleroga or rot disease. Mysore Agr. Dept. Mycol. Ser. Bull. **2**, 92 p.
3. Dade, H. A. 1928. A comparison of the pathogenicity of P. faberi Maubl. on cocoa pods, etc. in the Gold Coast. Gold Coast Dept. Agr. Yearbook **1927**: 89-92.
4. Dastur, J. F. 1916. Phytophthora sp. on Hevea brasiliensis. India Dept. Agr. Mem. Botany Ser. **8**: 217-232.
5. Demandt, E. 1918. Untersuchungen uber Kanker und Braunfaule am Samoanischen Kakao. Z. Pflanzenkrankh. **28**: 241-291.
6. Gadd, C. H. 1924. Phytophthora faberi Maubl. Ann. Roy. Bot. Gardens, Peradeniya. **9**: 47-89.
7. Gadd, C. H. 1927. The relationship between Phytophthora associated with the bud-rot disease of palms. Ann. Botany London **41**: 253-289.
8. Hartley, C. 1924. De Ziekten van de Cacao. Dutch East Indies. Department van landbouw, nijverheid en handel. Inst. v. Plantenziekten. Mededeelingen **19**, 16 p.
9. Leonian, L. H. 1930. Differential growth of Phytophthoras under the action of malachite green. Am. J. Botany **17**: 671-677.
10. Leonian, L. H. 1931. Heterothallism in Phytophthora. Phytopathology **21**: 941-955.
11. Leonian, L. H. 1934. Identification of Phytophthora species. West Va. Univ. Agr. Expt. Sta. Bull. **262**: 1-36.
12. Narasimhan, M. I. 1930. Studies in the genus Phytophthora in Mysore. I. Heterothallic strains of Phytophthora. Phytopathology **20**: 201-214.
13. Orellana, R. G. 1953. Infection and tissue changes of Theobroma cacao L. by Phytophthora palmivora Butl. Turrialba **3**: 167-172.
14. Orellana, R. G. 1954. Variation in Phytophthora palmivora isolated from cacao and rubber. (Abstr.) Phytopathology **44**: 501.
15. Reinking, O. A. 1923. Comparative study of P. faberi on coconut and cacao in the Philippine Islands. J. Agr. Research **25**: 267-284.
16. Rutgers, A. A. L. 1917. Hevea-Kanker. III. Dutch East Indies. Department van landbouw, nijverheid en handel. Lab. v. Plantenziekten. Mededeelingen **28**: 49 p.
17. Stoughton-Harris, R. H. 1926. Mycologist's Report for 1925. Rubber Research Scheme, Ceylon, Executive Committee Rept. **1925**: 22-25.
18. Thompson, A. 1929. Phytophtora species in Malaya. Malayan Agr. J. **17**: 53-100.
19. Tucker, C. M. 1931. Taxonomy of the genus Phytophthora de Bary. Missouri Univ. Agr. Expt. Sta. Research Bull. No. 153, 208 p.

COMBINATION REACTIONS OF THREE SMALL-GRAIN VIRUSES ON WHEAT [1]

Sardar B. Lal and Webster H. Sill, Jr.[2]

SUMMARY

Tests were based on visible differences of disease severity and on statistical studies of comparative dry weights of the above-ground portions of infected wheat plants. Brome mosaic virus (BMV) was typically the most severe virus alone and the dominant virus in combinations, followed by barley stripe mosaic virus (BSMV) and then by wheat streak mosaic virus (WSMV). Combinations of these viruses produced synergistic reactions of varying severity on wheat plants. WSMV + BSMV produced moderate to severe synergism. BSMV + BMV and the combination of all 3 viruses produced extreme synergism, the two reactions being statistically alike though each was significantly different from the single-virus controls. WSMV + BSMV + BMV reactions showed variations, as did the WSMV + BMV combination, and seemed to be controlled either by the severity of unknown strains of BMV in a given experiment or possibly by environment. BSMV and BMV symptoms dominated all reactions when in combination with WSMV. Except for BSMV in combination with BMV, the presence of individual viruses in all combinations was proved, showing that they actually did coexist in the same plant. When the second virus was introduced 2 weeks after the introduction of the first, the final virulence did not differ statistically from when they were introduced simultaneously. The standard extract of each virus was a 1:10 (plant-tissue–water) dilution by weight. Normally, 2 ml of each extract was mixed in the combination inocula, but some mixtures were made with one virus extract in as little as .05-ml amounts mixed with 2 ml of the other. Such mixtures produced synergism in some combinations but not in others, the severity of the reaction varying with different combinations having .05 ml of one virus. When 2 ml of one virus extract was mixed with 1 ml of another in preparing the inocula, the resulting synergism was like that occurring when equal volumes were used. Five strains of WSMV were tested, and each produced synergistic reactions of approximately equal severity when combined with BMV or BSMV, but no synergism occurred when they were combined with each other.

Interaction between unrelated viruses that simultaneously infect the same plant has been known for some time and has been discussed by Bennett (2) and Bawden (1). Such interaction has occasionally incited antagonism, as is typical with related strains of the same virus, and sometimes has been only additive in effect, but usually is synergistic.

McKinney (12), using only visual or qualitative comparisons of diseased plants, discussed synergistic reactions in dual infections with BSMV (barley stripe mosaic virus) and BMV (brome mosaic virus) on several varieties of winter and spring wheats and barley. Synergistic reactions also occurred when WSMV (wheat streak mosaic virus) was combined with BSMV or BMV in dual infections (13). Virulent or avirulent strains of WSMV did not seem to make any difference in the intensity of reactions, but dual infections were more severe when a virulent strain of BSMV was involved than when an avirulent strain was used.

During tests of samples of WSMV in the greenhouse in 1953, accidental mixtures with BMV produced symptoms on wheat much more severe than, and different from, those of either of the viruses alone. Investigations were undertaken by the junior author, beginning in the summer of 1954, to find out the nature of the reaction of the two small-grain viruses in combination. In 1955 a third virus, BSMV, was added in combinations with the other two viruses.

Both qualitative and quantitative methods were employed for comparing the severity of the various combination reactions. A preliminary account has been given of some of this work (4).

MATERIALS AND METHODS.—The viruses—WSMV, yellow streak mosaic strain (5), which is probably identical with McKinney's yellow streak strain (8); BSMV, virulent strain (17); and BMV (18), unknown strain or strains—used in this work were maintained in young Pawnee winter wheat plants in isolated cages in a greenhouse fumigated twice a week with Plantfume 103 (Plant Products Corp., Bluepoint, L. I., N. Y.) (active ingredient, 15% tetraethyl dithiopyrophosphate). The original source of these viruses was samples collected from the field in Kansas (6), diagnosed in the greenhouse with techniques described previously (16), desiccated over calcium chloride with a technique originally described by McKinney (9), and stored in a refrigerator at 1°C.

Young vernalized Pawnee wheat plants raised in 6-in. clay pots, 6 plants to a pot, were used as test plants. These were always inoculated at the 3-leaf stage to help assure uniformity of physiological development and reaction to the virus. Plants that failed to produce symptoms were discarded, and the 4 plants

[1] Accepted for publication November 12, 1958.
Contribution No. 517, Serial No. 670, Department of Botany and Plant Pathology, Kansas Agr. Expt. Station, Manhattan.

Combination infections of wheat streak mosaic virus and a Kansas collection of soil-borne wheat mosaic virus, probably wheat viruses 4 and 5 of McKinney (7), have also been observed for several years. Since no evidence of synergism has been seen, data are not included. The dominant virus has been WSMV under Kansas conditions, the combination reaction being essentially the same as WSMV alone.

[2] Assistant Instructor and Associate Professor of Botany, Kansas State College, Manhattan. A portion of a thesis submitted as partial fulfillment for the Ph.D. degree.

showing best symptoms were left in each pot and grown to maturity. All plants were supplied with fertilizer every 2 weeks (Kapco chemical fertilizer, 120 g per gal. water, manufactured by Summers Fertilizer Co., Inc., McKeesport, Pa.). Greenhouse experiments were conducted during the fall, winter, and spring. Temperatures were maintained at about 70°F during the winter, and were occasionally higher during the fall and spring. Artificial light was provided when needed to increase the day length and obtain better growth. The common carborundum leaf-wiping inoculation method for small grains (7) was used. All inoculum was plant tissue from young diseased Pawnee wheat plants diluted 1:10 by weight. The various combinations of the three viruses used in these tests were: 1) WSMV + BSMV, 2) WSMV + BMV, 3) BSMV + BMV, and 4) WSMV + BSMV + BMV. For simultaneous inoculation of 2 or more viruses, an inoculum consisting of 2 ml of each virus extract was mixed thoroughly. When the viruses were not introduced simultaneously, subsequent inoculations were delayed 2 weeks, giving the first virus enough time to establish itself before the second virus was introduced. Single-virus and healthy plant controls were always included in each experiment. Each set of inoculated plants or uninoculated controls consisted of 32 plants. Qualitative data were obtained by visual comparison of symptoms. Quantitative data consisted of comparative dry weights. The mature plants were harvested and washed, separated from roots, and dried in an oven at 90°C for 12 hours before being weighed. The quantitative data were statistically analyzed, using the analysis of variance followed by Fischer's LSD techniques (3).

EXPERIMENTAL INVESTIGATIONS.—*Simultaneous inoculations with equal volumes of component virus extracts.*—Experiments using Pawnee (C.I. 11669) and Triumph (C.I. 12132) winter wheats were conducted during the winter of 1955–56. Qualitative analyses of the reactions on Pawnee wheat were based on differential stunting of plants and general severity of the disease as exhibited by leaf symptoms, heading, and plant necrosis (Fig. 1). The single-virus controls showed that BMV (Fig. 1-D) was the most

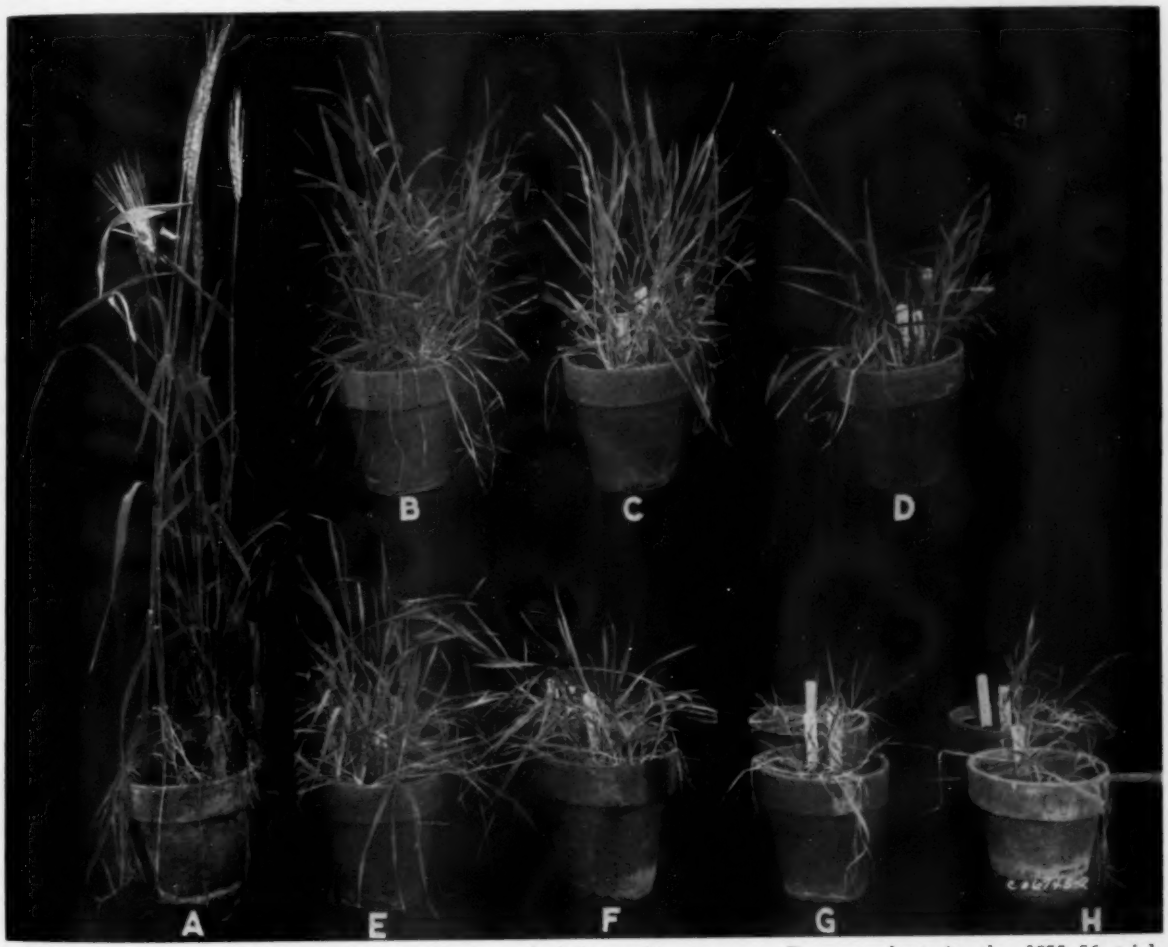


Fig. 1. Reactions of the 3 small-grain viruses and their combinations on Pawnee wheat in the 1955–56 trials. **A)** Uninoculated controls. The remainder are plants infected with **B)** wheat streak mosaic virus (WSMV), **C)** barley stripe mosaic virus (BSMV), **D)** brome mosaic virus (BMV), **E)** WSMV + BMV, **F)** WSMV + BSMV, **G)** BSMV + BMV, and **H)** WSMV + BSMV + BMV.

TABLE 1.—*Effect of simultaneous inoculation to wheat varieties with equal volumes of extracts of 3 small-grain viruses and their combinations as measured by dry weight of aboveground parts of diseased plants at maturity*

	Dry weight (g) of 32 plants				
	Winter 1955-56		Spring 1956		Winter 1956-57
Treatment[a]	Pawnee	Triumph	Lee[b]	Marquis	Pawnee
WSMV	71.6	59.7	11.9	16.6	148.7
BSMV	68.8	77.7	5.3	15.8	138.1
BMV	43.4	56.1	3.1	6.2	135.3
WSMV + BSMV	35.8	34.9	3.8	7.4	83.5
WSMV + BMV	33.5	40.6	3.3	4.1	44.5
BSMV + BMV	7.5	9.1	2.4	2.3	39.6
WSMV + BSMV + BMV	10.4	11.1	3.7	1.9	25.5
LSD at 5%[c]	10.6	11.4	2.3	2.6	34.1

[a] Inoculum for each virus was 1:10 dilution of extract from plant tissue in water (by weight). In the combinations, 2 ml of each virus extract were mixed.

[b] Var. Lee and Marquis were too susceptible for satisfactory use in the quantitative studies.

[c] Fischer's Least Significant Difference, at the 5% level, used here to make planned comparisons between treatments.

severe disease on Pawnee wheat under the greenhouse conditions used. This virus, first described by McKinney et al (14), induced light green mosaic and streaks in leaves of infected plants. Infected plants were occasionally killed. They tillered profusely, but few spikes were produced. Plants infected with BSMV (Fig. 1-C), also first reported by McKinney (10), were less stunted, had normal tillering, and produced more mature spikes. Leaf symptoms consisted of chlorotic mottling, spotting, and streaking. Grayish-white streaks or areas were intermixed with the light-green mosaic. Symptoms were often partially or completely masked in older plants. Diseased WSMV plants (Fig. 1-B), the detailed symptoms of which have been described earlier (8), were stunted least, often tillered profusely, and produced about as many spikes as BSMV-infected plants, though some were twisted and deformed. Infected leaves developed mosaic mottling and light-green streaks that coalesced and formed light-green to yellow patches, which often enlarged and gradually covered the whole leaf.

Plants infected with mixtures of BSMV and BMV (Fig. 1-G) and plants with all 3 viruses in combination (Fig. 1-H) exhibited the effects of an extremely synergistic reaction, resulting in the death of many plants. Plants that survived the initial shock reaction were extremely stunted, and rarely headed. Such plants had severe mosaic mottling, and leaves were often completely chlorotic. There was not much visual difference between plants infected with these 2 viruses and plants infected with all 3, although those infected with 3 were occasionally less severely damaged. The WSMV + BSMV (Fig. 1-F) combination produced yellowing of inoculated leaves. Young leaves had green to light-green mottling accompanied by white mosaic, often leading to complete chlorosis. Typical symptoms of BSMV tended to dominate, but the resulting reaction was much more severe than that produced by either of the viruses alone, indicating synergism. Infected plants produced many tillers, but usually failed to head. The reaction was less severe than that produced by the BSMV + BMV combination or by all 3 viruses together. The combination of WSMV + BMV (Fig. 1-E) produced a symptom pattern in which BMV seemed to dominate. The plants were usually stunted, but usually not more than by BMV alone, and headed sparsely or failed to head.

Triumph wheat plants infected with the single viruses and their combinations were usually somewhat more tolerant than Pawnee, and were not injured so badly as Pawnee by the more severe combinations, even though extreme synergism was obviously present. The variety Triumph has been reported by Pady et al (15) as tolerant to WSMV, and this may account for its relatively greater tolerance in the combinations that included WSMV.

The quantitative results for the 1955–56 trials are presented in Table 1. These were based on statistical differences between the dry weights of the aboveground parts of healthy controls, plants infected with each single virus, and each virus combination. The results were in general agreement with the visual differences already described. BMV was the most severe among single viruses on Pawnee wheat, being significantly different from WSMV and BSMV at the 5% level. The latter 2 viruses, when alone, were less severe, and did not significantly differ from each other. In combinations, the most severe reactions were produced by BSMV + BMV and WSMV + BSMV + BMV. The differences were significant when compared to their single-virus controls. These two combinations, however, did not significantly differ from each other. The reaction of WSMV + BMV was not significantly different from that of BMV alone or WSMV + BSMV. However, WSMV + BSMV produced a significantly more virulent reaction than did either of the single-virus controls. The two combinations WSMV + BMV and WSMV + BSMV were significantly less severe than the extremely virulent reactions produced by BSMV + BMV and by WSMV + BSMV + BMV.

The results with Triumph wheat (Table 1) showed the same order of severity, with a few minor exceptions. Instead of WSMV, BSMV was the least virulent virus alone, and its reaction was significantly less severe than that produced by either WSMV or BMV alone. The latter two did not differ significantly from each other in their virulence, but when combined produced significantly more severe reactions than either of the viruses alone.

The experiments were performed again during the spring of 1956, using Lee (C.I. 12488) and Marquis (C.I. 3641) spring wheats (Table 1). Visible differences in reactions were similar to those described for Pawnee except that at times the variations were

not so great. Marquis exhibited reactions like those shown by Pawnee, except that, in the quantitative study, WSMV + BMV did not differ significantly from the extremely severe reactions produced by BSMV + BMV and WSMV + BSMV + BMV. However, in this case, BMV alone was equally severe, and was also significantly more virulent than the WSMV + BSMV combination. On Lee wheat the pattern of reaction was generally the same. WSMV + BMV did not produce a significantly different reaction from that produced by BSMV + BMV, WSMV + BSMV + BMV, or BMV alone. The two spring wheats, actually too susceptible to show differences properly, did not prove to be good test plants.

Pawnee wheat proved to be the most favorable differential host in the 1955–56 trials. Hence, this variety was employed exclusively in the winter of 1956–57. Results were in general agreement with those obtained in the earlier tests (Table 1). Points of disagreement were as follows: The three viruses alone did not produce significantly different reactions from each other, being equally virulent (Table 1). The visual differences were similar (Fig. 2-BCD) except that BMV (Fig. 2-D) was comparatively less severe than in the 1955–56 trials. Also, in contrast to the preceding year, the reaction produced by WSMV + BMV was extremely virulent (Fig. 2-F). Many inoculated plants were killed, and the reaction was more severe than that produced by WSMV + BSMV or BMV alone (Fig. 2-ED). It was similar in these experiments to the extremely severe reaction produced by BSMV + BMV and WSMV + BSMV + BMV (Fig. 2-GH). The reaction produced by WSMV + BSMV + BMV looked slightly more severe than that of BSMV + BMV, but the two were not significantly different from each other.

Simultaneous inoculations with unequal volumes of component virus extracts.—The following combinations were tested on Pawnee wheat plants to determine whether unequal amounts of component virus extracts would influence the severity of the reactions. Two ml of each virus extract diluted 1:10 were thoroughly mixed with either 1.0 ml or .05 ml of the other virus extract, as shown in Table 2.

Both the qualitative observations and the quantitative data (Table 2) were in agreement. No symp-

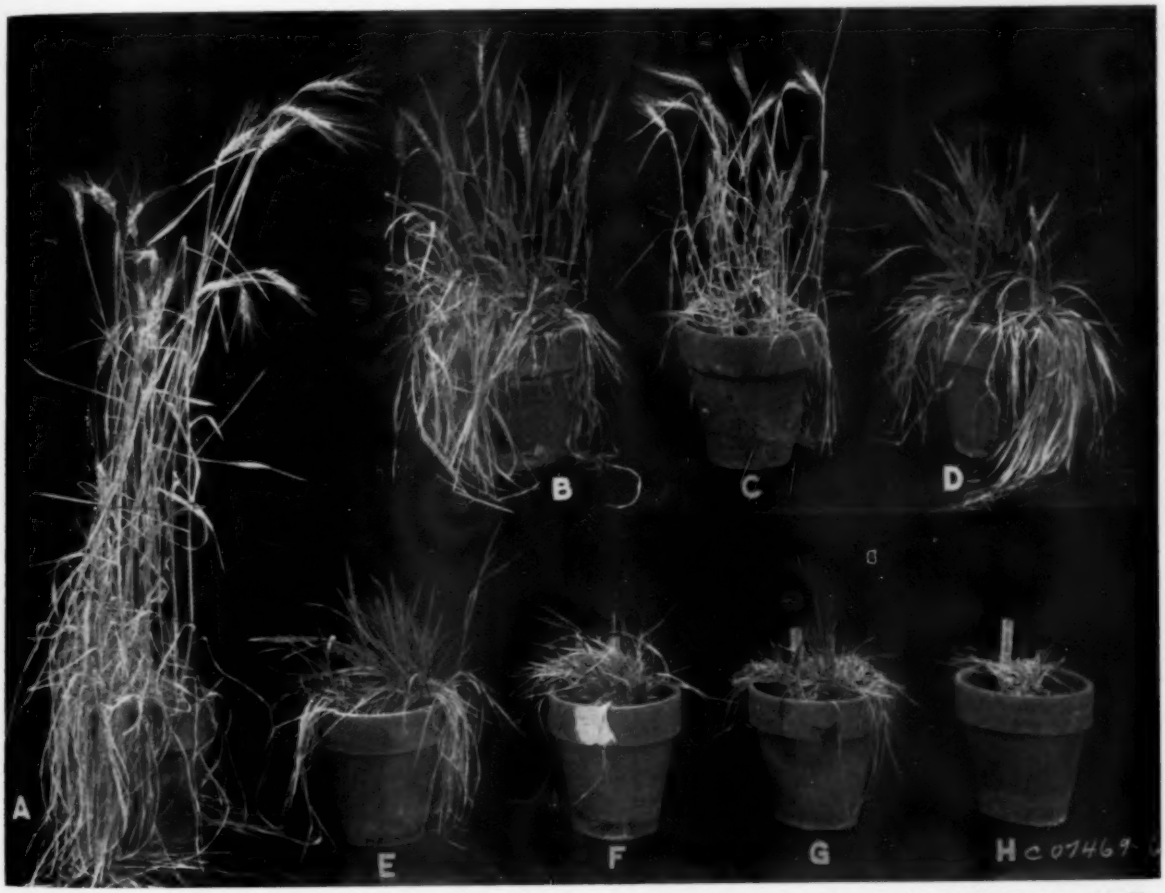


Fig. 2. Reactions of the 3 small-grain viruses and their combinations on Pawnee wheat in the 1956–57 trials. **A)** Uninoculated controls. The remainder are plants infected with **B)** wheat streak mosaic virus (WSMV), **C)** barley stripe mosaic virus (BSMV), **D)** brome mosaic virus (BMV), **E)** WSMV + BSMV, **F)** WSMV + BMV, **G)** BSMV + BMV, **H)** WSMV + BSMV + BMV.

tom or severity differences were evident between plants inoculated with a 2:1 ratio and those previously described that were inoculated with equal volumes of the virus extracts. All combination reactions produced synergism, the final reactions being significantly more severe than the reactions produced by either of the component viruses separately. In the BSMV + BMV combination, however, when 2 ml of either virus extract was combined with .05 ml of the other, the final reaction was significantly more severe than that of the single-virus BSMV controls, but not of the BMV controls. Reactions were usually significantly more severe from the 2:1 virus extract combinations than from the corresponding 2:0.05 combinations. An exception to this was the two combinations of BSMV + WSMV, both of which were equally virulent. In a combination of these it did not make any significant difference in the final reaction whether 2 ml of one virus extract was combined with 1 ml of the second virus extract, or the reverse. This was also true of the 2:0.05 combinations (Table 2). Among 2:0.05 combinations, BMV + WSMV and the reverse produced significantly more severe reactions than other combinations with one virus in .05-ml amounts. The combinations of BSMV + BMV and WSMV + BMV with 2 ml of the first virus extract combined with 1 ml of the second were significantly more severe than WSMV + BSMV, but not different from each other. All the above combinations containing BMV seemed to be dominated largely by the characteristic symptoms and reaction of that virus.

Inoculation with the second virus of the combination after systemic infection with the first virus.—In these experiments plants were inoculated with one virus first and were reinoculated with the other virus after 2 weeks. Plants inoculated with 1 virus alone served as single-virus controls. The leaf symptoms produced on the doubly inoculated plants were not different from those produced by the same combination of viruses inoculated simultaneously. There were slight visual differences in the severity of the two reactions, but these were not statistically different from comparable tests where the two viruses were inoculated simultaneously (Table 3). All reactions on doubly inoculated plants were, however, significantly more severe than their single-virus controls.

TABLE 2.—*The quantitative effect on Pawnee wheat of simultaneous inoculation with combinations of WSMV, BSMV, and BMV using unequal volumes of component virus extracts*

Treatments[a]	Dry weight[b] (g)
BSMV 2 ml + BMV 1 ml	4.2
BMV 2 ml + BSMV 1 ml	11.5
BMV 2 ml + BSMV .05 ml	54.5
BSMV 2 ml + BMV .05 ml	61.7
WSMV 2 ml + BMV 1 ml	8.8
BMV 2 ml + WSMV 1 ml	9.4
BMV 2 ml + WSMV .05 ml	30.9
WSMV 2 ml + BMV .05 ml	42.1
WSMV 2 ml + BSMV 1 ml	33.2
BSMV 2 ml + WSMV 1 ml	45.2
WSMV 2 ml + BSMV .05 ml	58.0
BSMV 2 ml + WSMV .05 ml	59.4
BMV alone	63.9
WSMV alone	99.1
BSMV alone	101.9
Healthy control	129.7
LSD at 5%[c]	15.8

[a] Each virus extract, before mixing with other viruses, was a 1:10 dilution (by weight) of plant tissue in water.
[b] Dry weights represent total dry weight of aboveground parts of 32 plants harvested at maturity.
[c] Fischer's Least Significant Difference, at the 5% level, used here to make planned comparisons between treatments.

TABLE 3.—*A comparison of the effect on Pawnee wheat of simultaneous inoculation of the 3 small-grain viruses and their combinations with inoculation of the individual viruses at 2-week intervals as measured by dry weight of aboveground parts of diseased plants at maturity*

Treatments	Dry weight (g) of 32 plants
Healthy control	197.3
WSMV alone	148.7
BSMV alone	138.1
BMV alone	135.3
BSMV : WSMV[a]	116.9
WSMV : BSMV[a]	84.1
WSMV + BSMV	83.5
WSMV : BMV[a]	71.1
BMV : WSMV[a]	56.9
WSMV + BMV	44.5
BSMV : BMV[a]	62.0
BMV : BSMV[a]	50.5
BSMV + BMV	39.6
LSD at 5%[b]	34.1

[a] Plants inoculated with first virus 2 weeks before inoculation with the second virus. All other combinations inoculated simultaneously.
[b] Fischer's Least Significant Difference, at the 5% level, used here to make planned comparisons between treatments.

Determination of the presence of the component viruses in infected plants.—The presence of individual viruses in plants infected with combinations was proved for BSMV and BMV by inoculating young seedlings of Golden Giant sweetcorn (16) and for WSMV by using the mite vector *Aceria tulipae* (K.) K. (19). All of the various combinations previously described were tested. Combinations of WSMV + BSMV inoculated to Golden Giant sweetcorn seedlings produced systemic infection characterized by broad white to yellow stripes, showing the presence of BSMV (16). Combinations of WSMV + BMV and of BSMV + BMV developed systemic infection and top necrosis that usually killed the seedling corn, showing the presence of BMV (16). The presence of BSMV in combination with BMV could not be detected with the techniques employed, because the BMV symptoms tended to dominate and mask BSMV symptoms on seedling corn. WSMV was isolated by the mite vector from all combinations with the other two viruses except when combined in .05-ml amounts. These tests may have failed because of the premature death of the plants under test. Seed transmission separation of BSMV (11) was not attempted. This was felt not to be necessary, because of the extreme

synergism typical of the combination reactions when compared with the single-virus controls.

Synergistic reactions of 5 strains of WSMV in combination with BSMV and BMV.—Extracts of each of 5 strains of WSMV (5) diluted 1:10 were mixed with equal amounts of 1:10 extracts of BSMV and BMV, and Pawnee wheat plants were inoculated with the mixtures. Twenty plants were inoculated with each of the 10 mixtures, with each of the five strains of WSMV separately, and with BMV and BSMV alone. Twenty plants were left as uninoculated controls. No statistical analyses were made, but the visual differences were similar to, and as great as, those analyzed and described previously.

All plants with BSMV in combination showed a synergistic reaction and severe yellowing. The leaves had light-green mottling mixed with white, and were considerably smaller than normal. Symptoms of BSMV dominated in each case. There was no killing, but the plants developed much more severe symptoms than the single-virus controls. There were no marked differences in virulence or symptoms produced by the different strains of WSMV in combination with BSMV.

Plants inoculated with BMV combined with each of the WSMV strains also exhibited severe synergism. Symptoms were a mixed yellow and white mottling and mosaic, with a tendency for leaves to be completely chlorotic. Plants were considerably stunted, and a few were killed or barely survived. The general reaction and symptoms of combinations were much more severe than their single-virus controls. The severity of the reaction was similar for all 5 strains of WSMV, and the symptoms of BMV dominated in each combination. The combination of any of the WSMV strains with BMV produced a more virulent reaction than the corresponding combination with BSMV, indicating again that BMV was the more severe virus on Pawnee wheat. Combinations of the 5 strains of WSMV with each other produced no synergism.

Discussion.—The choice of a satisfactory variety (or varieties) for a statistical study of this type is not usually easy to accomplish. Several wheat varieties were tested as possible hosts. The spring wheats Marquis and Lee proved to be too susceptible, the reactions of the single viruses and combinations being so severe that statistical differences were hard to demonstrate consistently. The winter wheat Triumph, although satisfactory, was somewhat more tolerant than desirable, and Pawnee winter wheat was finally chosen as the host that gave most satisfactory differences when inoculated with both the single viruses and the various combinations. All other varieties tested were discarded as unsatisfactory for various reasons.

Known strains of WSMV and BSMV gave consistent results, but the unknown strain (or strains) of BMV were variable, particularly when alone. However, when in any combination made up of equal volumes of the virus extracts BMV typically dominated; if BMV was not present, BSMV dominated. WSMV did not appear to be the dominant virus at any time in any combination. Since no strain studies of BMV have been reported, its behavior cannot be explained with any certainty at this time. The final virulence of the reactions was not affected by the time of inoculation of the combination viruses. In some experiments the viruses were inoculated simultaneously; in others, the different viruses were inoculated separately at biweekly intervals. There were slight visual differences as the symptoms developed, but no final statistically significant differences. These results may indicate that the first virus attacks different metabolic systems than the second or utilizes separate metabolites. If this be true, the time of inoculation of a given virus would be of minor significance to the final combination reaction.

When different amounts of virus extracts were used in the inoculated mixtures a certain minimum amount of each virus, which apparently differed for each combination, appeared to be necessary to cause synergism in the combination reactions, but as yet no techniques are known to the authors that could measure the exact minimal amount necessary. Studies such as this should be continued when it is possible to control minimal amounts more accurately, since they may give interesting clues concerning the mechanisms and meaning of synergism.

Although it was possible to prove the presence of the component viruses in a given plant by qualitative means, no satisfactory rapid methods for obtaining quantitative data for these 3 small-grain viruses have been reported. Hence, the relative increase and concentration of the component viruses in the combination-infected plants is unknown.

The synergistic reactions produced by the combinations of WSMV, BSMV and BMV on wheat plants indicated that the three viruses were not closely related to each other. By contrast, the five strains of WSMV tested appeared to be closely related, because they did not produce synergistic reactions when combined with each other in all possible combinations but did produce synergistic reactions when combined with BSMV or BMV. Thus, all tests pointed to either distant or no relationship between WSMV, BSMV, and BMV, and close relationship between strains of WSMV. The results also showed that the synergy reactions could be used for partial identification of these small grain viruses, as suggested by McKinney (13).

Department of Botany and Plant Pathology
Kansas State College
Manhattan, Kansas

LITERATURE CITED

1. Bawden, F. C. 1956. Plant viruses and virus diseases. Chronica Botanica Co., Waltham, Mass. 3rd ed. 335 p.
2. Bennett, C. W. 1953. Interactions between viruses and virus strains. Advances in Virus Research **1**: 39-67.
3. Fischer, R. A. 1949. Design of experiments. Oliver and Boyd, Edinburgh, London. 5th ed. 260 p.

4. LAL, S. B., AND W. H. SILL, JR. 1957. The behavior of combinations of three small grain viruses on wheat. (Abstr.) Phytopathology **47**: 22.
5. LAL, S. B., W. H. SILL, JR., MARIA S. DEL ROSARIO, AND J. M. KAINSKI. 1957. Three new naturally occurring strains of wheat streak mosaic virus in Kansas and the Great Plains. (Abstr.) Phytopathology **47**: 527.
6. LAL, S. B., AND W. H. SILL, JR. 1958. Stability of wheat streak mosaic virus in desiccated tissue. Plant Disease Reptr. **42**: 226-228.
7. MCKINNEY, H. H. 1937. Mosaic diseases of wheat and related cereals. U. S. Dept. Agr. Circ. 442. 22 p.
8. MCKINNEY, H. H. 1944. Descriptions and revisions of several species of viruses in the genera Marmor, Fractilinea and Galla. J. Wash. Acad. Sci. **34**: 322-329.
9. MCKINNEY, H. H. 1947. Stability of labile viruses in desiccated tissue. Phytopathology **37**: 139-142.
10. MCKINNEY, H. H. 1951. A seed-borne virus causing false-stripe in barley. (Abstr.) Phytopathology **41**: 563-564.
11. MCKINNEY, H. H. 1954. Culture methods for detecting seeed borne virus in Glacier barley seedlings. Plant Disease Reptr. **38**: 152-162.
12. MCKINNEY, H. H. 1956. Interference and synergistic reactions with respect to barley stripe-mosaic virus and brome mosaic virus. Plant Disease Reptr. **40**: 520-523.
13. MCKINNEY, H. H. 1956. Interference and synergy, their possible use in identifying certain mosaic viruses of cereals and indicating degrees of relationship. Plant Disease Reptr. **40**: 898-903.
14. MCKINNEY, H. H., H. FELLOWS, AND C. O. JOHNSTON. 1942. Mosaic of Bromus inermis. (Abstr.) Phytopathology **32**: 331.
15. PADY, S. M., C. O. JOHNSTON, W. C. HASKETT, W. H. SILL, JR., E. D. HANSING, H. FELLOWS, C. T. ROGERSON, AND J. C. FRAZIER. 1955. Diseases of wheat in Kansas. Kansas Agr. Expt. Sta. Bull. 368. 24 p.
16. SILL, W. H. JR. 1956. Use of corn and Agrotricum hybrids for more rapid identification of three grass viruses. (Abstr.) Phytopathology **46**: 26.
17. SILL, W. H. JR., AND E. D. HANSING. 1955. Some studies on barley stripe mosaic (false stripe) and its distribution in Kansas. Plant Disease Reptr. **39**: 670-672.
18. SILL, W. H. JR., AND R. C. PICKETT. 1955. Brome mosaic, a threatening grass virus disease. Plant Disease Reptr. **39**: 802.
19. SLYKHUIS, J. T. 1953. Wheat streak mosaic in Alberta and factors related to its spread. Can. J. Agr. Sci. **33**: 195-197.

VIRUS INCREASE IN SEEDLING ROOTS[1]

C. E. Yarwood[2]

SUMMARY

The viruses of tobacco necrosis, tobacco ring spot, sugar beet ring spot, sugar beet latent, peach yellow bud mosaic, and tomato bushy stunt increased in the roots of 4-day-old inoculated seedling beans in Petri dishes; some increased in the seedling roots of pea, corn, cowpea, or pine. Tobacco necrosis virus could not be recovered from the upper part of the hypocotyl or the tip of inoculated young bean roots, but was found in these tissues in greenhouse plants about 40 days after inoculation as seedlings. The concentration of tobacco necrosis virus doubled about every 4 hours at about 2 days after inoculation in 4-day bean seedlings. Sugar beet latent virus increased in association with seedling roots of bean, pea, corn, and cowpea, with or without prior injury of the roots; could be recovered from the diffusates from infected, turgid, uninjured roots; could be eliminated from the plants by surface sterilization of the roots with mercuric chloride; and disappeared in the roots of inoculated plants grown in soil. Several viruses did not increase in bean roots.

With several viruses, natural infection apparently is principally or only through the roots (3, 11). These and certain other viruses introduced into roots may move slowly or never to the tops of the plants. Yet studies of artificial inoculation of roots are relatively rare (2, 7), and the results erratic. It seemed likely that seedlings grown in Petri dishes would be suitable for studying inoculation methods and virus increase, and would have several advantages over greenhouse plants.

MATERIALS AND METHODS.—Seedlings of bean, pea, corn, cowpea, or pine were inoculated with the viruses of tobacco necrosis, tobacco mosaic, tobacco rattle, tobacco ring spot, sugar beet ring spot, peach yellow bud mosaic, tomato bushy stunt, tomato spotted wilt, alfalfa mosaic, Belladonna mosaic, cabbage black ring, carnation mosaic, and elderberry vein clearing, as well as with sugar beet latent virus and an unidentified virus from cowpea. Seeds were first germinated on moist filter paper in uncontrolled laboratory light, and inoculated 4–10 days later by dipping a finger in a water suspension of macerated diseased tissue and celite (Hyflo Super Cell, Johns Manville) and rubbing this on the seedling roots and hypocotyls. The inoculated seedlings were incubated at about 22°C on moist filter paper in Petri dishes in the laboratory for up to 10 days, but usually for only 5 days. No distinct symptoms appeared, and the inoculated roots or entire seedlings were macerated in water and assayed on appropriate local-lesion hosts in the greenhouse.

RESULTS.—With most virus-host combinations, no virus was recovered. These negative results are considered significant, but not conclusive, since demonstrable virus increase might result from more inoculations, longer incubation periods, different incubation

[1] Accepted for publication November 12, 1958.
[2] The work reported here was performed while the writer was a Guggenheim Fellow at the Virus Research Unit, Agricultural Research Council, Cambridge, England. Grateful appreciation to the Guggenheim Foundation and to Dr. K. M. Smith is hereby acknowledged.

environments, different strains of virus and host, or other experimental changes. The negative results will not be referred to in detail.

Most studies and most success were with tobacco necrosis virus (TNV) in French bean *(Phaseolus vulgaris)*, a combination that produces a natural and destructive disease in Holland (1). Of 71 Prince bean seedlings individually inoculated and assayed by standard methods in 5 tests, 57 became infected. Inoculations on seedlings were therefore reasonably successful, but not as successful as inoculations on leaves, where comparable inoculations would have resulted in infection on every leaf. To reduce failures in routine inoculation, 2 or more seedlings were commonly inoculated for each experimental unit, but in studies of method of inoculation, inoculations and assays were usually made with single seedlings.

Rate of virus increase.—Prince bean roots assayed 17 hours, 50 hours, and 71 hours after inoculation with TNV in the first trial yielded 0, 2, and 120 lesions, respectively. This gave a generation time (time for doubling the amount of virus) of 3.5 hours. Two other trials yielded 3.8 and 5.1 hours, averaging 4.1 hours. The average rate of increase is therefore considerably less than for TNV in leaves (14). However, the rate here is greater than for most plant viruses, and is quite adequate for most studies. In one trial, virus increase was detected 24 hours after inoculation.

Phosphate effect.—In previous studies (13), all virus inoculations (including TNV) to bean or cowpea leaves were greatly improved by adding K_2HPO_4 to the inoculum. In the present study, inoculations of bean roots with TNV without phosphate were apparently as successful as with phosphate. However, study of the phosphate effect was much less precise on roots than on leaves, since lesions could not be counted on roots, but an entire root was considered infected or uninfected on the basis of assay on bean or cowpea.

Site of inoculation.—Smith (9) has shown that TNV does not multiply in tobacco stems if inoculated there, though it multiplies readily in leaves or roots. Yarwood (15) reported that TNV and certain other viruses and fungi would not cause symptoms on bean pulvini. In the present study, bean seedlings were inoculated at various sites, and the entire seedlings were later assayed for virus. When the proximal (upper) region of the rootlet-producing zone was inoculated, virus could usually not be recovered in 1 day, but it could be recovered in moderate amounts in 2 days, and in relatively large amounts (50 or more lesions per assay bean or cowpea leaf from 1–20 dilution of bean root tissue) at 3 or more days after inoculation. Inoculation of the hypocotyl (the region between the cotyledons and the upper end of the rootlet-producing zone) was usually unsuccessful. Inoculations of the hypocotyl 2 cm above the rootlet zone of young seedlings were always unsuccessful; but within 1 cm of the rootlet zone, infection occurred in 2 out of 14 inoculations. The cotyledons were resistant to inoculations on either upper or lower surfaces. Inoculations of the tip cm of roots were successful in 1 of 6 seedlings.

For bean plants growing in pots, and therefore older than plants usually inoculated as seedlings, inoculation on plant parts aboveground usually yielded necrotic lesions. Virus could always be recovered from necrotic lesions, but usually not from inoculated areas showing no necrosis. Therefore, recognition of success of inoculation of plants growing in pots was usually based on the presence of necrotic lesions—a criterion that was not reliable in inoculations of roots. Partially expanded primary, true leaves were always susceptible, and the size of the lesions usually increased with the age of the leaves during the expansion stage. Hypocotyls of plants coming through the soil were usually resistant, but became increasingly susceptible with age, at least for the period about 6–15 days from seeding. Petioles and stems were distinctly more susceptible than hypocotyls of young plants, and frequently collapsed from infection. Pulvini, on the other hand, were occasionally susceptible on very young plants, but were usually resistant on plants older than about 10 days. In infections on hypocotyls, stems, petioles, or leaf blades, lesions extended from the point of inoculation, but usually stopped at the adjacent node. On hypocotyls the lesions usually did not progress below the soil line. Inoculations on petioles or laminae of leaves caused lesions that usually stopped at the pulvini, but the pulvini also became necrotic in 2 cases on very young plants.

Heating, shown to increase the susceptibility of beans to several viruses (16), was tested as a means of increasing the susceptibility of bean hypocotyls to TNV. In one trial where seedlings were heated 25 seconds at 50°C before inoculation, heat injury resulted and assay of the seedlings yielded 9 lesions on 2 cowpea leaves, whereas plants subjected to smaller amounts of heat did not yield infective inoculum. This trial was not repeated, but in 2 trials, heating cowpea leaves did not clearly increase their susceptibility to TNV.

Site of virus in infected tissues.—Smith (9) found that TNV did not normally move from tobacco roots to tobacco stems, and that the movement of TNV in bean may be stopped by a node. Similar results were secured here. Virus was usually recovered from the same tissue through which inoculation of seedlings was successful. Tissues that were resistant to inoculation were also resistant to invasion of the virus from other parts. When the rootlet zone was successfully inoculated, virus was recovered principally from the rootlet zone, not from the hypocotyl or root tip. Virus was sharply localized in the upper part of the rootlet zone, even though the entire hypocotyl and root had been inoculated. In 2 trials, 6 seedlings were inoculated on roots and hypocotyls 6 days after seeding and assayed 6 days after inoculation. Tissue was divided for assay into the region 6–10 mm above the secondary roots, 3–6 mm above the secondary roots,

0–3 mm above the secondary roots (these 3 samples are regarded as hypocotyl), 0–3 mm below the upper limit of secondary roots, 3–6 mm below the upper limit of secondary roots, and 6–10 mm below the upper limit of secondary roots (these 3 samples are regarded as root). Respective average numbers of assay lesions per cowpea leaf for the 6 regions were 0.2, 3.1, 89, 109, 78, and 33. Agreement between trials was good except for the region 6–10 mm below the upper region of secondary roots, where the lesions per leaf were 0.25 in one trial and 66 in the other.

Abrasion and rubbing.—Bean seedlings inoculated by dipping momentarily in a virus suspension or by adding drops of virus suspension to the roots did not become infected with TNV, whereas roots inoculated by rubbing with TNV plus celite usually became infected.

Inhibition by water.—Tomato roots in liquid culture were not infected by rubbing with tobacco mosaic virus, but virus multiplied in roots taken from infected plants and grown in liquid culture (12). It has been suggested that failure to infect roots in water might be caused by inhibition of infection by water. To test this, germinating bean seedlings were inoculated with TNV in the ordinary way. One set was incubated in Petri-dish moist chambers where the only source of water was that used to wet the filter paper in the dish. Other lots of inoculated seedlings were immersed in distilled water for periods up to 18 hours and then incubated 3 days in a moist chamber. In most cases, immersion in water appeared to decrease infection, but results were inconsistent; and even after 18 hours of immersion, 3 of 8 seedlings were infected. It appears that immersion in water is less inhibitory to infection of bean roots by TNV than to infection of bean leaves by tobacco mosaic virus.

TNV increase in pine seedlings.—Seeds of *Pinus sylvestris*, germinated on moist filter paper in the laboratory, mostly produced seedlings about 2 cm long and were considered suitable for inoculation about 10 days after seeding. For virus assays within 10 days of inoculation, inoculated seedlings were held in moist chambers in the laboratory; for assays made 10 days or more after inoculation, inoculated seedlings were immediately transferred to pots of soil in the greenhouse. The seedlings held in the laboratory were so small that the roots of 10 or more were used for each assay; 3 seedlings were used for each assay of plants in soil. Five or more cowpea plants were used as local-lesion indicator plants for each assay.

Uninoculated, control pine seedlings, tested only twice, yielded no assay lesions on cowpea. In 5 independent series of inoculations and 9 independent assays, virus was recovered from roots plus hypocotyls in 4 assays. One assay each at 2, 4, 14, 27, and 54 days after inoculation yielded no virus. One assay at 5 days yielded 2 lesions, one at 8 days yielded 11 lesions, one at 10 days yielded 48 lesions, and another at 10 days yielded 208 lesions (on 15 cowpea plants). Though generalizations from such limited data are unsafe, it appears that TNV increase can be detected in pine roots as soon as 5 days after inoculation, reaches a maximum in about 10 days, and disappears from plants in soil in 14 or more days. This apparent disappearance of TNV in pine roots with age or for some other reason is not considered unique, for the same type of disappearance of TNV has been detected in lettuce roots.

The increase of TNV in pine roots is not used here as evidence in support of the generalization that TNV has a wide host range and might be expected to multiply in almost any roots, for no increase of this strain of this virus was detected in the roots of pea, corn, cucumber, or carrot.

Sugar beet latent virus (SBLV).—This infective agent (10), not yet conclusively shown to be a virus, behaved differently from TNV in several respects. Inoculations on bean roots were more reliable than with TNV, and all 45 Prince bean seedlings inoculated with SBLV became infected. SBLV attained high titers on seedlings of bean, cowpea, cucumber, pea, and corn, on commercial roots of beet and carrot, and on pear fruits, whereas TNV apparently did not increase in these last 6 hosts. However, SBLV could not be recovered from roots of bean, pea, cucumber, and corn that had been inoculated as 4-day seedlings, then planted in soil, and assayed 34 days later, though it was recovered in similarly inoculated bean roots—in large amounts 2 days after planting in soil, and in small amounts 4 days after planting in soil. SBLV was recovered from the hypocotyls, rootlet zone, and root tips of Prince bean, and from the leaves of dent corn. SBLV caused infection when drops of virus suspension were added to bean roots in Petri dishes, or when roots were dipped in virus suspension, but not as regularly as if the roots were rubbed with virus. Bean plants dipped in 0.1% $HgCl_2$ for 10, 50, 250, and 1250 seconds at 4 days after inoculation with SBLV and then washed and assayed yielded no virus, whereas similarly treated plants that were infected with TNV yielded only slightly less virus than seedlings that were not surface-sterilized. SBLV, like TNV, showed no increase in infection due to K_2HPO_4 in root inoculations, but, unlike all other viruses tested (13), showed no response to K_2HPO_4 when inoculated on cowpea leaves. Immersing TNV-infected roots in water for 12 hours did not confer infectivity on the immersion liquid, as revealed by assay, but immersing SBLV-infected bean roots in water for 12 hours produced a suspension that yielded many lesions on cowpea.

Other viruses that increased in seedling bean roots but have not been studied intensively are sugar beet ring spot virus, peach yellow bud mosaic virus, tobacco ring spot virus, and tomato bushy stunt virus.

Discussion.—The use of seedlings for virus multiplication is not new. Posnette and Strickland (6) used dissected cacao bean seeds as substrata for feeding infective mealy bugs in the transmission of swollen

shoot virus. Swollen shoot virus could not be transmitted mechanically, but when the virus was transmitted to seeds by mealy bugs, symptoms of the disease appeared 17–25 days later. By the previous standard technique, 10- to 12-month-old seedlings had been inoculated, and symptoms appeared in a further 17–69 days.

In the present study, symptoms were not observed in the seedlings, but, with TNV, virus increase was detectable by assay 2 days after inoculation.

On the basis of these preliminary results, it is suggested that multiplication of viruses in seedlings in Petri dishes has several advantages over the use of entire plants in greenhouses. Ease of inoculation of roots with viruses may be correlated with natural infection via roots. With seedling roots in Petri dishes, the interfering effect of soil, with its many microorganisms, is eliminated or reduced. Roots on germinating seeds are suitable for inoculation in about 3–10 days after seeding, whereas leaves of potted plants are usually available in about 8–40 days. The multiplication of virus in total darkness, which is normal for roots, eliminates the necessity for light and greenhouses and permits studies of viruses in controlled environments in standard incubators; whereas, for growing plants in greenhouses, the environment is usually rather variable under the best-controlled conditions available at most institutions. Most environmental factors, such as light, temperature, and humidity, can be more reliably studied on plant roots than with potted plants. In combination with virus assay by serology (5), multiplication of plant viruses in roots can be studied without greenhouse or outdoor facilities. Use of seedlings permits more experimental units per unit of space than is possible with regular greenhouse culture.

Seedling roots have some of the advantages of tissue cultures, plus some additional advantages. The rate of virus increase observed here, and the success of inoculations on roots, is greater than that observed in tissue cultures of tobacco mosaic virus (4). The time required to establish seedling root cultures is less than for tissue cultures, and the rate of increase of seedling roots is much greater.

The serious disadvantage of virus culture in seedling roots in these tests has been that the roots usually did not show symptoms of infection, and had to be assayed on local-lesion hosts.

Department of Plant Pathology
University of California
Berkeley 4, California

LITERATURE CITED

1. Bawden, F. C., and J. P. H. van der Want. 1949. Bean stipple-streak caused by a tobacco necrosis virus. Tijdschr. Plantenziekten **55**: 142-150.
2. Fulton, Robert W. 1941. The behavior of certain viruses in plant roots. Phytopathology **31**: 575-598.
3. Harrison, B. D. 1957. Studies on the host range, properties, and mode of transmission of beet ringspot virus. Ann. Appl. Biol. **45**: 462-472.
4. Kassanis, B., T. W. Tinsley, and Frederika Quak. 1958. The inoculation of tobacco callus tissue with tobacco mosaic virus. Ann. Appl. Biol. **46**: 11-19.
5. Matthews, R. E. F. 1957. Plant virus serology. Cambridge Univ. Press.
6. Posnette, A. F., and A. H. Strickland. 1948. Virus diseases of cacao in West Africa. III. Technique of insect transmission. Ann. Appl. Biol. **35**: 53-63.
7. Roberts, F. M. 1950. The infection of plants by viruses through roots. Ann. Appl. Biol. **37**: 385-396.
8. Smith, K. M. 1937. Studies on a virus found in the roots of certain normal-looking plants. Parasitology **29**: 70-85.
9. Smith, K. M. 1951. Recent advances in the study of plant viruses. 2nd ed. Churchill, London. 300 p.
10. Smith, K. M. 1951. A latent virus in sugar-beets and mangolds. Nature **167**: 1061.
11. Smith, K. M. 1957. A textbook of plant virus diseases. 2nd ed. Little, Brown, Boston. 652 p.
12. White, P. R. 1934. Multiplication of the viruses of tobacco and aucuba mosaics in growing excised tomato root tips. Phytopathology **24**: 1003-1011.
13. Yarwood, C. E. 1952. The phosphate effect in plant virus inoculations. Phytopathology **42**: 137-143.
14. Yarwood, C. E. 1952. Latent period and generation time for two plant viruses. Am. J. Botany **39**: 613-618.
15. Yarwood, C. E. 1954. Resistance of bean leaf pulvini to fungi and viruses. Phytopathology **44**: 64.
16. Yarwood, C. E. 1956. Heat-induced susceptibility of beans to some viruses and fungi. Phytopathology **46**: 523-525.

STUDIES ON THE CONTROL OF CITRUS CANKER WITH STREPTOMYCIN[1]

G. Rangaswami, Rao Rama Rao, and A. R. Lakshmanan

SUMMARY

Streptomycin sulfate, when sprayed in aqueous solutions together with 1% glycerin on acid lime (*Citrus aurantifolia*), was found to be absorbed by the leaves better than when sprayed alone. Indications were obtained for the presence of the antibiotic in the leaf sap of 3-year-old citrus plants within 12 hours of spraying, and also for its persistence up to 21 days after spraying. Streptomycin sulfate at 1000 and 500 ppm sprayed at 15-day intervals effectively checked citrus canker, caused by *Xanthomonas citri*, but did not do so at 250 and 100 ppm. There were indications to show that the canker bacterium was killed *in vivo* by antibiotic spray at high concentrations.

The seriousness of citrus canker, caused by *Xanthomonas citri* (Hasse) Dowson, can be measured by the costly eradicative measures that have been undertaken against the disease in the United States and in South Africa. While eradication has proved successful in these two areas, canker continues to be a problem wherever complete eradication has not been practiced. In India, where the disease is said to have originated (6), it is found wherever citrus is grown. At present, recommended control measures consist of burning all trees found affected. Naturally, this works considerable hardship in a land where the disease is already widespread. Checking the disease with chemical sprays like Bordeaux mixture has not been found satisfactory. Streptomycin, the most successful of the antibiotics in plant disease control, was tested against citrus canker.

The inhibitory effect of streptomycin on plant-pathogenic species of *Xanthomonas*, and the use of this antibiotic in controlling diseases, have been reported by several workers (1, 2, 4, 5, 7, 9, 10). Rangaswami (11) recently reported on its effect on *X. citri;* streptomycin sulfate was found to check *in vitro* the growth of the bacterium at 1 μg/ml. The present report describes field and laboratory studies on the control of citrus canker with streptomycin sulfate.

MATERIAL AND METHODS.—The experiments were conducted in the University Orchard at Annamalainagar, Madras State. A block of 40 3-year-old acid lime plants (*Citrus aurantifolia* (Christm.) Swingle) were selected for spray trials. The experiment was laid out in a randomized block design with 5 treatments and 8 replications.

A pharmaceutical grade of streptomycin sulfate (Glaxo Laboratories) with 740 units/mg was used for spraying the plants in all experiments. Aqueous solutions of the antibiotic were prepared using tap water and glycerin added to obtain 1% concentration. The mixture was sprayed on the citrus plants with a Primus-type pressure sprayer. All treatments were carried out at the same time, either in the morning or in the evening.

The presence of the antibiotic inside the host tissue was tested by the leaf-disk and agar-cup assay methods (3) using *Bacillus subtilis* Cohn emend. Prazmowski (ATCC 6633) as the test organism in nutrient agar medium. For the leaf-disk assay, 1-cm disks were taken from the central region of the leaf. For the agar-cup assay, sap was obtained by grinding washed leaf tissues with the addition of 1 ml sterile distilled water. The assay plates were incubated 24 hours at room temperature before observations were recorded.

From each of the plants in the experiment, 3 shoots were selected and labeled. The intensity of canker infection of leaf from the 3 shoots was recorded as 0 = no infection, E = 10%, D = 11–30%, C = 31–50%, B = 51–75%, and A = >75%, using a grading chart. Periodical observations were made on the incidence and spread of canker on each of the shoots, and the aggregate disease index for each plant was obtained by assigning the category values of 0–5, respectively, for the above-mentioned disease intensities of 0 to A.

EXPERIMENTAL RESULTS.—*Absorption of streptomycin by citrus leaves.*—Gray (8) reported that the addition of 1% glycerin to spray increased the effectiveness of streptomycin against the common blight of beans. To test glycerin in the control of citrus canker, potted acid lime plants were sprayed with 500 ppm of aqueous streptomycin sulfate, with and without 1% glycerin. The leaves from the sprayed and check plants were collected after 4, 8, and 24 hours, and assayed for the antibiotic by the leaf-disk assay method, using *B. subtilis* as the test organism. In all cases, adding glycerin increased the absorption capacity of the citrus leaves. Because of these results, the subsequent streptomycin sprays were invariably mixed with 1% glycerin.

Spray trials in the field.—Spray trials were conducted in the field during February–June, 1958. Before each spray treatment, individual plants were examined and the intensity of canker infection recorded. In all, the plants were sprayed 7 times at 15-day intervals, except for the period April 5 to May 4, 1958, which was used for studying the persistence of the antibiotic in plant tissues. The results obtained are summarized in Table 1.

Results show that streptomycin sprays at 1000 and 500 ppm effectively checked the spread of the canker. The statistical analyses of the data obtained after

[1] Accepted for publication November 12, 1958.

TABLE 1.—*Effect of streptomycin spray on the spread of citrus canker in the field*

Treatment	Dates observed (1958)*							
	2/19	3/6	3/21	4/5	5/4	5/19	6/3	6/18
Check: 1% glycerin	2.9	1.8	2.9	3.4	3.0	3.7	4.4	4.6
100 ppm strep. sulfate + 1% glycerin	3.0	1.4	2.8	3.8	2.8	3.5	3.9	4.1
250 ppm strep. sulfate + 1% glycerin	3.1	1.5	2.4	3.6	2.8	3.6	4.0	4.1
500 ppm strep. sulfate + 1% glycerin	3.0	0.4	0.6	0.5**	0.5	0.6	0.6	0.8**
1000 ppm strep. sulfate + 1% glycerin	3.0	0.0	0.0	0.1**	0.1	0.1	0.3	0.3**
Standard error				0.182				0.259
LSD 1%				0.710				1.020

* Average values for 8 plants on the following scale: 0 = no infection, 1 = 10% infection, 2 = 11–30% infection, 3 = 31–50% infection, 4 = 51–75% infection, 5 = >75% infection.
** Differences significant at 1% level.

the third and seventh sprayings indicate that the 2 concentrations are significantly better than the rest of the treatments; streptomycin sulfate at 250 and 100 ppm seems ineffective in checking the spread (Table 1). There was a sudden drop in the disease index of plants sprayed with 1000 and 500 ppm streptomycin, whereas, in treatments with 250 and 100 ppm, progress of the disease was parallel to that in check plants (Fig. 1).

During April–May a large number of water shoots appeared on all plants. These shoots grew very fast and were subsequently found infected by the bacterium, the infection being uniform in all the plants under trial. When the leaves from these shoots were assayed against *B. subtilis* in the usual manner, there was no inhibition of the bacterium in any of the treatments, indicating that the antibiotic in the older leaves was not translocated into the rapidly growing

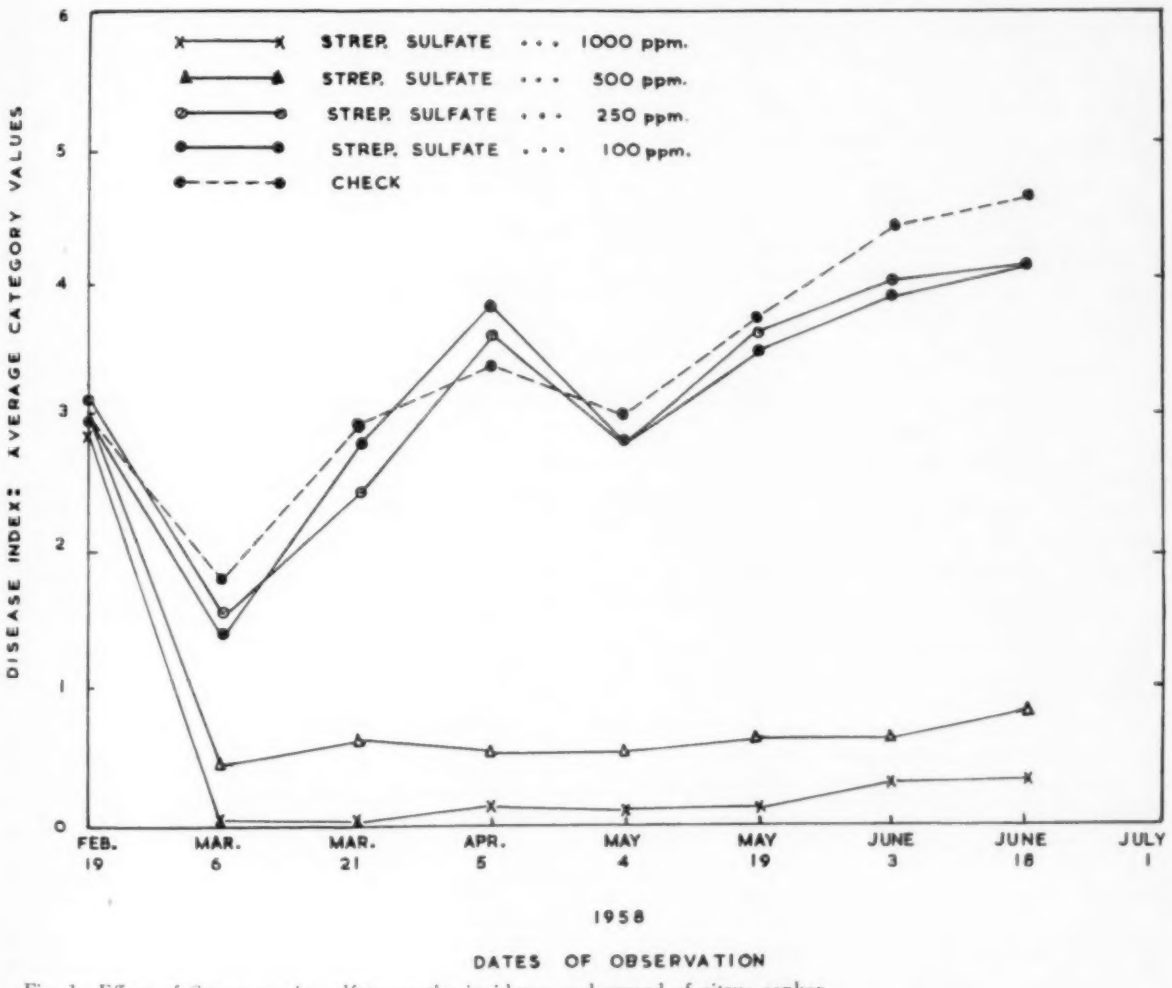


Fig. 1. Effect of Streptomycin sulfate on the incidence and spread of citrus canker.

TABLE 2.—*Persistence of streptomycin sulfate in citrus leaves*

Treatment	Diameter of inhibition zone (mm) after (days)[a]					
	½	1	8	15	21	30
1000 ppm strep. sulfate + 1% glycerin	12	16	20	16	12	0
500 ppm strep. sulfate + 1% glycerin	0	12	16	12	12	0
250 ppm strep. sulfate + 1% glycerin	0	12	12	11	0	0
100 ppm strep. sulfate + 1% glycerin	0	12	12	0	0	0
Check: 1% glycerin	0	0	0	0	0	0

[a] Agar-cup assay in nutrient agar medium.

tissues. Subsequent sprays with higher concentrations of streptomycin sulfate, however, checked the spread of disease on water shoots also.

Persistence of the antibiotic in leaf tissues.—Leaves from the treated plants were assayed at intervals by the agar-cup assay method. The results are summarized in Table 2.

Presence of the antibiotic in the sap was indicated by its inhibitory property against *B. subtilis*. There was relatively more inhibition with the sap obtained from the plants sprayed with higher concentrations than with the lower concentrations. The inhibitory property of the sap seems to be reduced with time, there being no inhibition after a month, even in sap from plants sprayed with 1000 ppm of the antibiotic.

Phytotoxicity of streptomycin sulfate on citrus.—Toxic symptoms, in the form of circular chlorotic spots on the leaves, were observed within 24–48 hours after treatment on plants sprayed with 1000 and 500 ppm of the antibiotic, but not at lower concentrations. These chlorotic spots, 4–10 mm in diameter, were found more commonly on the younger leaves than on the older ones. With age, the chlorotic symptoms disappeared in most cases. During the first and second sprayings, relatively more spots appeared on the leaves than after subsequent sprayings. After the seventh and final spray, practically no chlorotic symptoms were observed, indicating that the plants ceased to react adversely to the antibiotic treatment.

In vivo effect of streptomycin sulfate on X. citri.—Within 24 hours of spraying with 1000 and 500 ppm of streptomycin sulfate, canker spots on the leaves were dried up, there being no water-soaked appearance even in the young ones. On the other hand, in the plants treated with 250 and 100 ppm as well as in check plants, there was no apparent change in the general symptoms of the canker spots. Attempts were made to isolate the bacterium from canker spots collected from the various treatments. Though the bacterium could be easily isolated from plants treated with 250 and 100 ppm, several attempts failed with plants treated with 1000 and 500 ppm, indicating that the bacterium had been killed by the higher concentrations of antibiotic.

DISCUSSION.—In the present field experiments the plants for the various treatments were selected at random. The highly infected check plants formed a good basis of inoculum for the rest of the plants under the experiment. If all the plants in the orchard are sprayed with antibiotic at the same time, it would no doubt result in more effective checking of the disease.

The studies on the persistence of the antibiotic in the leaves show that the spraying is to be done at least once in 15–21 days to be effective in preventing fresh infections, and spraying at shorter intervals would probably be necessary during seasons when rapidly growing water shoots appear on the plants. Periodic removal of these water shoots may also help, by maintaining plants with more-resistant foliage on them. The cost of spraying antibiotics at such short intervals will be prohibitive to many orchardists. Studies to evolve an economic and effective spray schedule for the plants and to test cheaper and more effective agricultural preparations of streptomycin should be conducted before any recommendations are made.

DEPARTMENT OF AGRICULTURE
ANNAMALAI UNIVERSITY
ANNAMALAINAGAR, MADRAS, INDIA

LITERATURE CITED

1. ALTMAN, J., AND B. H. DAVIS. 1955. Field tests with streptomycin nitrate for control of bacterial leaf spot of pepper. Phytopathology **45:** 183.
2. CONOVER, R. A. 1954. Control of bacterial spot of tomato and pepper seedlings with Agrimycin. Plant Disease Reptr. **38:** 405-409.
3. CROWDY, S. H., AND D. PRAMER. 1955. Movement of antibiotics in higher plants. Chem. & Ind. London **7:** 160-162.
4. DAINES, R. H. 1956. Bacterial spot of peach (Xanthomonas pruni) and its control by the use of captan and streptomycin. Plant Disease Reptr. **40:** 335-336.
5. DUNEGAN, J. C. 1954. Antibiotics in plant disease control. J. Agr. Food Chem. **2:** 1020-1022.
6. FAWCETT, H. S., AND A. E. JENKINS. 1933. Records of citrus canker from herbarium specimens of the genus Citrus in England and the United States. Phytopathology **23:** 820-824.
7. GARBER, J. D., J. W. ROTHROCK, H. C. REYNOLDS, AND R. A. GRAY. 1955. Recent laboratory and greenhouse studies with agricultural streptomycin. Antibiotics Ann. 1954-55. p. 530-535.
8. GRAY, R. A. 1955. Increasing the effectiveness of streptomycin against the common blight of beans with glycerin. Plant Disease Reptr. **39:** 567-568.
9. HAGBORG, W. A. F. 1956. Effect of antibiotics on infection of wheat by Xanthomonas translucens. Can. J. Microbiol. **2:** 80-86.
10. MARLATT, R. B. 1955. Effectiveness of streptomycin as a control for common bacterial blight of Pinto bean. Plant Disease Reptr. **39:** 213-214.
11. RANGASWAMI, G. 1957. Development of resistance to streptomycin in Xanthomonas citri and X. malvacearum. Current Sci. India **26:** 185-186.

REPORT AND ABSTRACTS OF THE 1958 ANNUAL MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 18th Annual Meeting of the Northeastern Division of the American Phytopathological Society was held at West Springfield, Mass. on November 6 & 7, 1958. Approximately 165 people were in attendance. Nineteen scientific papers were presented and several informal progress reports on the results of the 1958 fungicide tests were made.

The officers elected for 1959 were as follows: *President*, A. E. RICH; Vice President, J. E. LIVINGSTON; Secretary-Treasurer, D. E. MARSDEN; Councilor, E. M. STODDARD.

J. E. LIVINGSTON, *Secretary-Treasurer*

Dutch elm disease control with polychlorobenzoic acid. BECKMAN, C. H. Evidence has indicated that treatment of trees at bud-break with chemicals that 1) are growth inhibitors, 2) penetrate bark, and 3) are mobile within the host might control the Dutch elm disease. Therefore, 2,3,5,-6-tetrachlorobenzoic acid (65% in oil, Amchem Products Co.) was selected for field experiments. Twenty-five elms 12 to 18 feet tall were treated by painting a 2-inch-wide band of the polychlorobenzoic acid with or without the chloride salt of calcium, magnesium, manganese, or iron (500 ppm) around the base of the trunk during bud-break (May 9). Similarly, 25 trees were treated at full-leaf (May 22). Each of these and 20 untreated trees were inoculated with a composite conidial suspension of *Ceratocystis ulmi* (Buis.) Moreau, on June 23 and again on July 18. Incidence of infection by September 13 was 95% of untreated trees, 52% of all trees treated at full leaf, and 16% of all trees treated at bud-break. Infection was lowest (none) and injury was minor (8% foliar necrosis) when polychlorobenzoic acid was used without salts at bud-break. These results support the proposed criteria for selection of control agents.

The role of tobacco mosaic virus in the internal browning disorder of tomato. BOYLE, JOHN S. Seventeen isolates of tobacco mosaic virus (TMV), 1 isolate of tobacco ring-spot virus (TRV), 1 isolate of cucumber mosaic virus (CMV), and 1 isolate of an unidentified virus from *Mertensia virginica* (L.) Link. were used to inoculate 40 plants each of the tomato var. Rutgers grown in the field in replicated and randomized 10-plant plots. One hundred twenty plants of the variety Rutgers served as non-inoculated controls. Final records on the severity and incidence of internal browning in all treatments were taken on an individual plant basis by harvesting and cutting all fruit beginning 21 days after inoculation. The highest incidence of IB in any one treatment of the TMV-isolate block was 97.5% (39 of 40 plants producing internally browned fruit), with 20.94% (593 affected of 2832) of all fruit produced showing symptoms. By comparison, the incidence of IB in the controls was 17.5% (7/40), with 0.36% (9/2498) fruit affected; 28.95% (11/38), with 3.91% (98/2508) fruit affected; and 0% (60/39), with 3021 unaffected fruit. The incidence of IB in the plants inoculated with CMV, TRV, and the virus from *Mertensia* was not significantly different from that in the controls.

Row treatment of soils infested with Plasmodiophora brassicae. CETAS, R. C. Sassafras loam and sandy loam soils naturally infested with *Plasmodiophora brassicae* Wor. were given row treatments of VPM (31% sodium methyldithiocarbamate). In greenhouse tests, the fumigant was dribbled into open furrows that were closed immediately. Chisel applicators were used in field trials. The VPM was injected 6 in. deep. A water seal was applied immediately. The amount of water applied varied with the moisture content of the soil. Cabbage plants set 14–30 days later were not injured. The treatment of warm (70°F or above) dry soils with 1 pt. of VPM per 100 ft. of row, followed by the application of 1.5 in. of water by overhead irrigation, resulted in a weed-free band about 16 in. wide, excellent clubroot control, and satisfactory plant growth in the field. In greenhouse tests conducted under similar soil conditions, the use of 0.5 pt. of VPM per 100 ft. of row followed by a 0.5-in. water seal resulted in satisfactory control of clubroot and of weeds in an 8-in. band. In contrast, the injection of 1 pt. of VPM per 100 ft. of row into cold (55°F) loam soils saturated with water resulted in no or very poor control of weeds, and unsatisfactory control of clubroot, but an increase in plant growth.

A tractor-mounted field-plot sprayer. CETAS, R. C., AND R. E. PARTYKA.

Stem and leaf blight of birdsfoot trefoil caused by Fusarium roseum. FORD, R. E. Birdsfoot trefoil (*Lotus corniculatus* L.) seedlings showing symptoms of leaf and stem blight have been observed in New York fields. Diagnostic symptoms are bleaching and drooping of stem tips, eventually followed by death of the whole shoot. *Fusarium roseum* Snyd. & Hans. was isolated consistently from plants showing these symptoms. Symptoms produced by plants inoculated with pure cultures of the organism were the same as those observed on plants in the field, and *F. roseum* was reisolated. Optimum temperature for disease development at 100% relative humidity was near 82°F. Inoculum prepared by fragmenting cultures in a blender was more infective than was that consisting of spore suspensions. Wounding is not essential for infection. Seedlings of *L. corniculatus*, 1–3 weeks old, were not susceptible to a root rot caused by this organism, but 3 related species (*L. arenarium* Brot., *L. divaricatus* Boiss., and *L. maroccanus* Ball) exhibited severe root rot 3 weeks after having been transplanted into steam-sterilized soil infested with *F. roseum*.

Relationship of loss of pectolytic enzyme synthesis to avirulence of induced mutant strains of a soft rot bacterium. FRIEDMAN, B. A., AND M. J. CEPONIS. *Pseudomonas marginalis* (Brown) Stevens, the causal agent of a soft rot of head lettuce and witloof chicory, forms protopectinase, pectin depolymerase, and pectinmethylesterase in culture. Avirulent isolates were obtained following UV irradiation. Five of these avirulent isolates, selected at random, failed to produce detectable pectolytic enzymes in culture, whereas the parent strain did. The mutants have been transferred on nutrient agar at about biweekly intervals for a period of 6 months but have shown no reversion to a virulent state. Attempts to induce the production of pectolytic enzymes in 1 avirulent strain by supplementing the culture medium with unheated pectin were unsuccessful. This isolate also failed in fermentation tests to produce alkali from sodium pectate or sodium polygalacturonate or acid from sucrose as the sole carbon source in synthetic media. In other respects the avirulent strain and the parent strain had identical morphological, cultural and physiological characteristics. It is concluded that the loss of ability to synthesize pectolytic enzymes by some of the radiation-induced mutant isolates of *Ps. marginalis* is genetic in nature and the cause of their loss of virulence.

Bark-patch grafting over tree pruning wounds. HOLMES, F. W. Decay has been commonly observed after prolonged exposure of wood in pruning wounds. If this wood is covered promptly with a layer of wound paint or of living marginal callus tissue, decay organisms are excluded.

However, paints crack or peel, and callus growth is slow. In an effort to prevent entrance of fungi more effectively, patches of elm bark were grafted over pruning wounds in elm trees. Best results were obtained when 1) both patch and wound were shaped by cutting around the same spindle-shaped 20-gauge galvanized sheet-tin template, fitted to the tree, 2) the patch was first dipped in an aqueous solution containing 100 ppm 3-indoleacetic acid, 3) the patch was applied with the same orientation in which it originally grew, and 4) mild pressure was applied over the surface of the patch. Where all of these conditions prevailed, the patch tissues merged with the host tissues around the entire periphery of the wound, and the patch formed a layer of wood 2.5–4.5 mm thick under its whole surface between June 20 and September 13. No evidence of decay was observed in this wood.

Control of Alternaria leaf blights and storage rots of sweet Spanish onions. HUSAIN, S. M., AND A. G. NEWHALL. Onions in small replicated plots were sprayed with a power sprayer during 3 seasons. In 1956, best leaf blight control was obtained with Dyrene (50% 2,4-dichloro-6-(*o*-chloroanalino) triazine) at 5-day intervals (8 applications), followed closely by Oleocuivre (40% Cu_2O) and maneb (70%), nabam (22%), Iron Vancide (70% ferbam + iron salt of 2-mercaptobenzothiazole), captan (50%), and griseofulvin (95% at 200 ppm), in that order. A 10-day interval (4 applications) was not so satisfactory. Yields were generally correlated with leaf blight control, but total storage rots caused by *Alternaria* sp., *Botrytis* sp., *Fusarium* sp., and bacteria were not. In 1957, Dyrene, Oleocuivre, and maneb at 2 and 4 lb./100 gal. (200 gal./acre) were compared on a 5-day schedule. The 2× concentration was not better than 1×. Dyrene and maneb controlled blights better than Oleocuivre. Only Dyrene and Oleocuivre reduced Botrytis storage rot significantly at the 1% level. In 1958, Dyrene, maneb, and Oleocuivre were superior, in that order, to thiram (42% water suspension), ziram (76%), and Cyprex (70% dodecylguanidine acetate) in the control of leaf blights. Dyrene, Oleocuivre, and maneb have thus far been more effective against blights (and the first 2 against Botrytis rot) than the other 7 fungicides.

A reaction between captan and the dialkyldithiocarbamates. LUKENS, R. J. Captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) reacts with Na-dimethyldithiocarbamate (Na-DDC) by a 2-stage reaction to give tetramethylthiuramdisulfide (TMTD), tetramethylthiurammonosulfide(TMTM), tetrahydrophthalimide, CS_2, and NaCl. Four moles of Na-DDC are needed to decompose completely 1 mole of captan. TMTM and CS_2 probably form from an unstable addition product of thiophosgene and 2 DDC ions after the initial reaction of captan with Na-DDC. The toxicities of captan:NaDDC (1:4 molar ratio) reaction mixtures to *Saccharomyces pastorianus* Hans. are the same as equivalent amounts of Na-DDC alone. In 4 days, aqueous suspensions of $Zn(DDC)_2$ (ziram) and $Fe(DDC)_3$ (ferbam) react with captan, but the rates are much lower than that of Na-DDC. If captan is used in a spray schedule with ziram or ferbam, either simultaneously or in an alternating schedule, this reaction may occur with the formation of TMTD and TMTM in the spray residue.

Cobalt pretreatment of yeast cells increases toxicity of captan. LUKENS, R. J., AND S. RICH. Pretreatment of cells of *Saccharomyces pastorianus* Hansen with $CoCl_2$ ($10^{-4}M$) and washing them free of excess cobalt makes them twice as susceptible to poisoning by captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) as are washed cells not pretreated with cobalt. Cells treated with cobalt alone grow just as well as controls. $ZnSO_4$ ($10^{-4}M$) is also synergistic with captan in toxicity, but to a lesser degree than cobalt. The following compounds have no demonstrable effect on the toxicity of captan to the growth of *S. pastorianus:* $CuCl_2$, $NiCl_2$, Na-nitroprusside, iodobenzoic acid, iodoacetic acid, and p-chloromercuribenzoate. Cobalt-treated cells release ½ the amount of CS_2 from captan ($3\times10^{-5}M$) as the controls. The CS_2 detected is that freed from superficial areas of the cell. Hence, pretreatment with cobalt apparently reduces the amount of captan destroyed at the surface or in the outer regions of yeast cells. Apparently, cobalt binds non-vital sulfhydryl groups at or near the surface of yeast cells, permitting more captan to penetrate to deeper, vital sites. Presumably, a large proportion of fungicide entering fungus cells may be detoxified by reactions not vital to the cell.

Plant viruses seen west and east of the Iron Curtain. MARAMOROSCH, KARL.

Control of gray mold on strawberries. MILLER, P. M., AND E. M. STODDARD. At the first picking, quadruplicated 10-ft plots of Stelemaster strawberries averaged 109, 89, and 39 *Botrytis-cinerea*-infected berries, respectively, when sprayed 1, 2, or 3 times with Dichlone (50%) ⅜ lb/100 gal. Similar plots averaged 115, 63, and 26 infected berries, respectively, when sprayed 1, 2, or 3 times with thiram (65%) 3 lb/100 gal. Plots sprayed 3 times with captan (50%) 6 lb/100 gal. averaged 55 infected berries, and the unsprayed plots averaged 93 infected berries. Counts were made 14 days after the third application. Comparing only fruit sprayed 3 times, the least gray mold developed on the harvested fruit from thiram plots both 1 and 4 days after picking. Differences among treatments were less on the fourth day and also 1 day after the next picking, 7 days after the first. Sparkle berries sprayed 5 times with thiram, dichlone, or captan ranked in the same order as in the Stelemaster plots. Again the differences were greater in the first picking and less evident at another picking 8 days later. Sprays did not lower yields of either variety. All sprayed Stelemaster plots yielded approximately 5000 g per 10-ft plot, and unsprayed plots yielded 4000 g per 10-ft. plot.

Systemic infection of bean by tobacco necrosis virus in New York. NATTI, JOHN J. A naturally occurring systemic infection of bean plants (*Phaseolus vulgaris* L.) observed in the field and greenhouse at Geneva, N. Y., was found to be caused by a tobacco necrosis virus. Symptoms produced on experimentally inoculated plants indicated the disease to be closely related, if not identical, to stipple streak, a virus disease of bean that has been observed only in Holland. In greenhouse tests, all of 67 varieties of *P. vulgaris* L. tested and one variety of *Glycine Max* Merr. (Manchu) became systemically infected as a result of inoculation of primary leaves. Local lesions, but no systemic infection, occurred on *P. lunatus* L. (Henderson's bush and Fordhook 242). No symptoms were observed on, and virus was not recovered from, inoculated leaves of *P. coccineus* L. (scarlet runner and white Dutch runner), *Lotus corniculatus* L., *Medicago sativa* L., *Melilotus alba* Desr., *Pisum sativum* L., *Trifolium hybridum* L., *T. incarnatum* L., *T. pratense* L., and *T. repens* L. Local necrotic lesions were produced on a wide variety of other plant species. In virus-infested soil, 13% of the bean plants (California red kidney) from direct seeding, and 52% of transplanted bean seedlings developed systemic infection.

Evidence of quack grass toxicity to apple seedlings. PALMITER, D. H. Potted apple seedlings watered with a cold-water extract of quack grass (*Agropyron repens* L.) rhizomes failed to grow normally. One half of the treated trees died within 60 days of the time the treatment started. Plants that survived were rosetted and averaged about 1/10 the size of control plants. Some of the leaves on treated plants became chlorotic, and later showed necrosis. An equal number of control seedlings received no extract and grew normally. Other apple seedlings were grown in a liquid medium containing quack grass extract. These plants developed symptoms similar to those of treated trees in pots. The roots did not appear to be injured by the extract, but the leaves became chlorotic and the top growth was stunted. Roots of control seedlings (no extract) were soon attacked by various fungi. These fungi

did not develop where the extract was used, indicating its toxicity to fungi as well as to apple seedlings. The nature of the toxic substance leached from quack grass rhizomes has not been determined.

The effect of nitrogen, phosphorus and potassium levels on growth and virus content of excised tobacco-mosaic-virus-infected tomato roots. PAPASOLOMONTOS, A., AND R. E. WILKINSON. The nutritional requirements of excised tomato roots from one plant, both healthy and infected with tobacco mosaic virus (TMV), were investigated by measuring their growth in modified White's culture solutions. In normal White's solution, diseased roots were shorter and produced fewer laterals than did healthy roots. When the level of N or P was increased to a point that caused stunting of healthy roots, however, the diseased roots were as long as or longer than the healthy roots. For N and P, therefore, the requirements of TMV-infected roots appear to be higher than those of healthy roots. Differences in the K requirement were not apparent. The virus activity of the TMV-infected roots was assayed on *Nicotiana glutinosa*. Virus concentration was found to increase as the level of N or P in the culture solution was increased from sub-normal to above-normal amounts. The effect of various concentrations of K was not very marked, and was not statistically significant. The results are in accord with the theory that the systems that produce normal plant protein and virus protein compete for available N and P. Both systems appear to function best when there is an ample supply of these nutrients to alleviate this competition.

Transmission of barley yellow dwarf virus acquired from liquid preparations by aphids feeding through membranes. ROCHOW, W. F. Nonviruliferous English grain aphids (*Macrosiphum granarium* Kirby) were placed in a feeding chamber that was topped with Silverlight capping skin membrane. Aphids were allowed to feed through this membrane for about 17 hours on liquid virus preparations. They were then transferred to healthy California Red oat seedlings (5–10 aphids per seedling) for a 3-day test feeding. In general, crude virus extracts were prepared by grinding infected plants in cold 10% sucrose. Source plants were oats or barley infected with New York barley yellow dwarf virus strains that are regularly transmitted efficiently by this vector from plant to plant. Various kinds of preparations were used in the 24 experiments completed. Some virus transmission occurred in each experiment, but the amount of transmission varied greatly among tests. In 9 tests with crude sucrose extracts, for example, the numbers of test plants infected (over the numbers of plants infested) were 4/6, 1/6, 6/15, 9/12, 17/24, 3/16, 5/18, 4/16, and 17/47. Controls in these 9 tests consisted of 108 comparable test seedlings infested with aphids from the 9 colonies used; none became infected.

A possible cause of tobacco fleck. TAYLOR, GORDON S. The sporadic, overnight collapse of palisade cells in maturing leaves of certain shade-grown tobacco varieties produces irregular brown or white spots called "weather fleck." Fleck is worse in direct sunlight and along open rows, field edges, and the southwest sides of plants and fields. It does not appear under overlapping leaves or on tissues distal to a cut midrib. It is reduced by mist-blown sprays containing either 0.71% maneb or 0.17% dichlone plus 0.73% cottonseed oil, and by 16.5% zineb dust. Fleck usually appears in the field after the intercellular leaf spaces have been flooded by rain, fog, or heavy dew. The cellular pattern of water congestion in the field often exactly matches the fleck pattern that subsequently develops. Water congestion alone, however, does not cause fleck. Symptoms identical to fleck can be induced by gassing leaves with SO_2. Evidently, direct sunlight predisposes tissues to threshold damage by air-borne toxicants. Weather-produced flooding of such tissues causes death of the palisade cells, possibly by asphyxiation.

Reliability of the detached-leaf technique for testing alfalfa clones for resistance to black stem. WARD, C. H. There is little information available on the reliability of the detached-leaf technique for testing alfalfa clones for resistance to black stem caused by *Ascochyta imperfecta* Pk. Seventy-two clones of differing degrees of resistance were used for comparative tests in the field, greenhouse, and laboratory. Each was replicated 4 times at each location. Inoculum was 21-day-old agar cultures fragmented in a blender containing water. In the field, plants were inoculated 3 times and disease records made 10 days after the last inoculation. Plants in the greenhouse were inoculated with an atomizer, placed in an incubation chamber for 3 days, and then returned to the greenhouse. Disease severity was recorded 7 days after inoculation. Leaves were detached and taken to the laboratory, where they were washed with water, atomized uniformly with inoculum, and floated on 2% sucrose solution. Disease records were made on detached leaves 4 or 5 days after inoculation. Disease records did not differ greatly, as indicated by correlation analyses, when the same clones were inoculated in the field or in the greenhouse, or when leaves detached from the clones were inoculated in the laboratory. For this reason, the detached-leaf technique is considered reliable.

PHYTOPATHOLOGICAL NOTES

Fusaric Acid in Banana Plants Infected with Fusarium oxysporum f. cubense. O. T. PAGE. Two principal mechanisms have been proposed to account for symptom expression in fusariose vascular disease: 1) pathogen-induced tracheary obstructions that cause a water shortage and loss of turgor,[1] and 2) systemic vivotoxins that inhibit certain plant functions, such as respiration.[2] Both mechanisms are apparently operative in the Panama disease syndrome incited by *Fusarium oxysporum f. cubense* (E. F. Sm.) Snyd. & Hans. Vessel plugging and an imbalanced water economy have been demonstrated in Fusarium-infected banana plants.[3]

The present paper reports the isolation of FA (fusaric, or fusarinic, acid) from cultures of *F. oxysporum* f. *cubense*, and from rhizomes of Gros Michel banana plants infected with this fungus. The phytotoxic complicity of FA in several other fusariose syndromes, and the mode of formation of this acid *in vitro*, have been reviewed by Gäumann.[4]

Preliminary to chromatographic determination of whether FA was present in culture filtrates and rhizome extracts, a known sample of FA (melting point 106–107° C) was chromatographed. FA (5*n*-butylpyridine-2-carboxylic acid) has been reported to chelate such metal ions as Cu^{++} to form stable inner metal (cupric) complex salts.[2] Although the cupric-FA chelate structure yields a blue color, this color was not sufficiently sensitive for chromatographic identification of the complexed FA. A color reaction sensitive to *ca* 1.0 μg FA resulted when rubeanic acid (dithiooxamide) was combined with cooper. In this reaction the di-imido form of rubeanic acid forms a colored inner metal complex salt with the copper in the copper-FA chelate compound. Accordingly, FA, 0.005*N*, was applied in 10-μl aliquots to Whatman No. 1 filter paper, dried, and complexed *in situ* with 0.02*N* $CuSO_4$, added in 10-μl aliquots to the FA spots. Chromatograms were equilibrated for 4 hours in an atmosphere saturated with respect to the aqueous phase of a 4:1:5 (by volume) mixture of *n*-butanol, acetic acid, and water. Development of chromatograms by descending irrigation with the butanol phase of the solvent mixture was continued for at least 18 hours at 22°C. After drying, chromatograms were sprayed with 0.1% rubeanic acid in acetone. The resultant copper rubeanate was detected as an olive-gray spot; the R_f value was 0.93.

Preliminary to identifying FA in culture filtrates, a monoconidial isolate of *F. oxysporum* f. *cubense* was grown in darkness at 30°C on a medium consisting of 10.0 g NH_4NO_3, 5.0 KH_2PO_4, 2.5 g $MgSO_4 \cdot 7H_2O$, 20.0 mg $FeCl_3 \cdot 6H_2O$, 50.0 g d-glucose, and 1000 ml distilled water. After incubation the culture medium was filtered to remove mycelium, and the filtrate, adjusted to pH 4.0, was extracted with ethyl acetate. The ethyl acetate extract was then dried *in vacuo* at 40°C and the residue taken up in 95% ethanol. Unknown samples complexed with copper were chromatographed alone, and in combination with known FA.

FA from filtrates was chromatographically identical to the known sample of FA. A calculated yield of about 10 mg of FA per liter of filtrate, produced by the pathogen after 12 days of incubation, suggests that FA is a product of rapidly growing mycelium rather than a product of lysis. With longer incubation, 20-fold yields of FA have been obtained from culture filtrates under similar conditions of substrate and temperature.

To demonstrate the presence of FA in diseased Gros Michel banana plants, tissue samples were taken from infected rhizome stele and the juice expressed. Yield was about 100 ml per 400 g fresh weight of tissue. The juice was centrifuged 10 minutes at 3200 rpm, filtered, and adjusted to pH 3.5. Aliquots of 100 ml of acidified juice were extracted 10 times with 10-ml amounts of chloroform, and the combined chloroform extracts were dried in a flash evaporator at 30°C. The extract residue soluble in 95% ethanol was chromatographed alone and with known FA; all samples were complexed with copper.

Certain difficulties are inherent in the detection of FA in diseased plants. The acid forms chelate compounds with metal ions within a plant, chelating with greatest intensity with Fe^{+++}. Diseased-rhizome extracts were therefore expected to contain several metal complex salts of FA. Adequate chromatographic separation of known cupric and ferric fusarates was not obtained by irrigating with water, or HCl-saturated butanol, with several solvent mixtures, or on NaCl-impregnated paper. Separation of cupric fusarate in known samples, to permit identification as derived cupric rubeanate, was obtained by development with collidine and water (1:1, by volume). The R_f was 0.0 for the ferric complex, and 0.80 for the cupric complex.

Quantitative estimation of FA in plant extracts by chromatographic methods, whereby FA is detected as metal complex salts, is subject to substantial error. Such error results from the degree of dissociation of FA, a function of pH, and the relative amounts and chelation intensity of certain metal ions present in the host extract. The method of quantitative estimation of FA in culture filtrates will be presented elsewhere.—Department of Plant Pathology, Vining C. Dunlap Laboratories, Tela Railroad Co. (A subsidiary of United Fruit Company), La Lima, Honduras.

[1] Dimond, A. E., and P. E. Waggoner. 1953. The water economy of Fusarium wilted tomato plants. Phytopathology 43: 619-623.

[2] Tamari, K., and J. Kaji. 1954. Studies on the mechanism of the plant-growth inhibitory action of fusarinic acid, a toxin produced by Gibberella fujikuroi Wr., which is the causative mould of the Bakanae disease of rice plants. Faculty of Agr. Bull. 6 Niigata Univ., Niigata, Japan.

[3] Page, O. T. 1959. Observations on the water economy of *Fusarium*-infected banana plants. Phytopathology 49: 61-65.

[4] Gäumann, E. 1957. Fusaric acid as a wilt toxin. Phytopathology 47: 342-357.

The Distribution on Nicotiana glutinosa Leaves of Lesions Produced by Tobacco Mosaic Virus.[1] IRVING RAPPAPORT AND SAM G. WILDMAN. When lesions appear on *Nicotiana glutinosa* L. inoculated with TMV, a great many may appear in some leaf areas and none in others. To determine whether distribution reflects inoculation technique or variations in intrinsic sensitivity over the leaf surface, the following experiment was performed.

Three commonly employed inoculation techniques were compared: the brush, the glass paddle, and the index finger. With each method, a dilution series of TMV in Celite was made so that the distribution of lesions could be analyzed and compared over a large range. The maximum number of lesions appearing per leaf was about 1000. During inoculation, each leaf was supported on a bed of wet absorbent cotton molded to the leaf's under surface. During the leaf rubbing, great care was taken to ensure as uniform and complete coverage with the inoculum as possible. Leaves were left on the plants until the lesions appeared, and then removed. The petiole and major portion of the midrib were cut away before a leaf was affixed with Scotch tape, to the uncarboned side of a sheet of carbon paper, which was then placed over a sheet of cm-graph paper. The center of each lesion was pierced with a fine needle so that an impression from the carbon appeared on the graph paper. The outline of the leaf was then traced. The total area of the leaf was arbitrarily divided into squares of 25 mm^2. Each square was counted, and the number of lesions in each was recorded. It was assumed that, if the lesions appeared at random, the data would conform to a Poisson distribution.

It was soon apparent that the lesions were not distributed at random. Certain areas contained many more lesions than expected, and others remained free of lesions regardless of method of inoculation. As lesion numbers increased, the difficulty of infecting certain areas became more obvious. The departure from randomness is illustrated in the accompanying figure, where the ratio of the observed zero class to the expected, e^{-n}, is plotted as a function of n, the average number of infected foci per 25 square mm. The data suggest that, at the time of inoculation, there was an intrinsic variation in sensitivity to infection on the leaf. Areas more difficult to infect required a greater concentration of TMV than easily infected areas. Furthermore, certain areas may be entirely resistant to initial infection. Areas that appear least sensitive are those near the petiole and near the leaf tip. The data do not distinguish between such models as a non-random distribution of equally susceptible sites, a random distribution of sites with a non-random susceptibility to infection, or any combination of these.—Department of Botany, University of California, Los Angeles 24.

[1] Supported, in part, by research grant, E1190, National Institutes of Health, U. S. Public Health Service, and contract AT (11-1)-34, Project 8, U. S. Atomic Energy Commission.

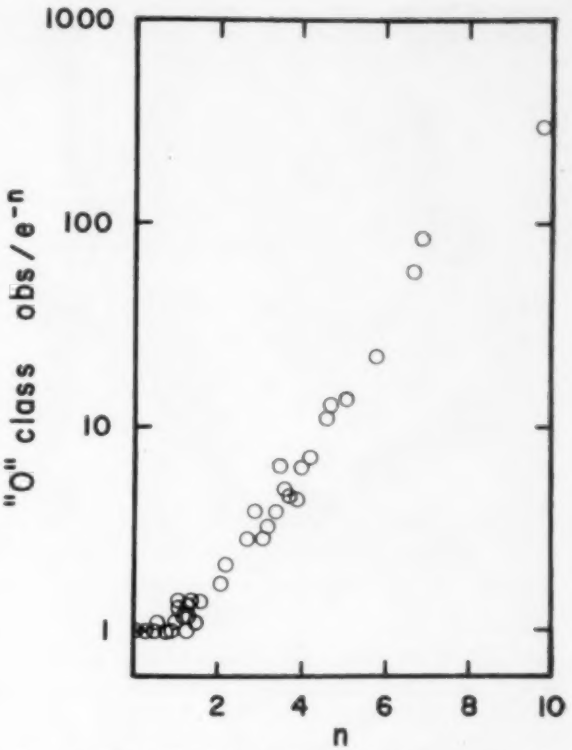


FIG. 1. The difficulty experienced in infecting certain areas of *Nicotiana glutinosa* leaves, expressed as a function of the average number of lesions, n, per 25 mm^2 unit area.

Use of Cellophane Tape for Mounting and Photographing Phytopathogenic Fungi. EDWARD E. BUTLER AND MARGERY P. MANN. As discussed by Carmichael,[1] Cellophane has been used in various ways as an aid in the study of fungi. Cellophane tape, on the other hand, has received little mention as an aid in such studies. Acetate tape has been used for collecting medical fungi from infected surfaces, and its use has been suggested for examining phytopathogenic fungi.[2]

In this laboratory, Cellophane tape has proved excellent for removing fungi, such as the downy mildews, from infected plant surfaces, and for routine examination of many fungi growing on agar media or other substrates. The technique is as follows: A strip of tape 0.75 in. wide (about 2.0 cm) and about the length of a glass slide is held taut with a thumb and forefinger at each end, with the adhesive side on the forefingers. One thumb is free to move to the center of the strip and press the tape gently onto the surface to be examined. A slide with a drop of lactophenol is used for mounting. The tape is fastened to

[1] Carmichael, J. W. 1956. The Cellophane technique for studying morphology and hyphal fusions in fungi. Mycologia 48: 450-453.

[2] Edwards, R. W., and E. Hartman. 1952. A simple technique for collecting fungus specimens from infected surfaces. Lloydia 15: 39.

Fig 1. Conidia of *Alternaria tenuis sensu* Neergaard.[3] ×500.

one end of the slide, drawn tightly and evenly over the drop of lacto-phenol, and sealed to the glass at the other end. The mount is ready for microscopic examination.

Cellophane-tape mounts have the advantage of retaining spores attached to sporophores and intact chains of conidia in the case of hyphomycetes, such as *Alternaria tenuis* (Fig. 1). Some definition is sacrificed, for Cellophane tape is inferior to glass in optical qualities.

Lacto-phenol appears to be the best mounting fluid. Water is satisfactory for short periods, but it eventually reacts with the adhesive, forming a cloudy film.

In mounting fungi on Cellophane tape for photographs, the following procedure gives excellent results (Fig. 1). The fungus is removed from a substrate as previously described and, with the aid of one or two dissecting needles, placed, adhesive side up, on a clean slide. One end of the mounting tape is then fixed to the slide with a piece of tape about 3 cm long, placed at right angles to the long axis of the slide. A similar short piece is fastened in like manner to the other end of the mounting tape, which is then pulled absolutely flat against the slide and fastened in place by the second short length of tape. Excess tape is removed with a razor blade. A drop of lacto-phenol and a cover glass are then placed over the fungus. Avoid excess lacto-phenol, for mounts are unsatisfactory if it flows under the tape.

Alternaria tenuis (Fig. 1) and *Stemphylium botryosum* have been photographed. This method should give good results with many other fungi.—Department of Plant Pathology, University of California, Davis, California.

[3] Neergaard, P. 1945. Danish species of Alternaria and Stemphylium. E. Munksgaard, Copenhagen. 560 p.

BOOK LISTINGS

KLINKOWSKI, MAXIMILIAN, ed. and 8 others. 1958. **Pflanzliche Virologie, Volume 1, Einführung in die allgemeinen Probleme.** Academie-Verlag, Berlin. 279 p., 103 illustrations. Price, about $12.00.

A complete textbook of plant virology consisting of chapters on symptomatology, means of transmission, insect vectors, host relationships, biophysics and biochemistry of plant viruses, morphology, serology, variability, classification and nomenclature, means of detection, and means of control. The last 46 pages constitute a laboratory manual in which experiments for students of the subject are outlined and discussed.

KLINKOWSKI, MAXIMILIAN, ed. and 6 others. 1958. **Pflanzliche Virologie, Volume 2, Die Virosen des europäischen Raumes.** Academie-Verlag, Berlin. 393 pages, 251 illustrations. Price, about $8.50.

A handbook of the viral diseases that have been reported as occurring in Europe. The volume is unusually well illustrated and printed on paper of excellent quality. An extensive list of German, Latin, and English common names for host and test plants is presented. A final section deals with virus-transmitting aphids and their synonyms. The subject matter of the entire volume is adequately indexed.

GRAINGER, THOMAS S. 1958. **A guide to the history of bacteriology.** Ronald Press, New York, N.Y. 210 p. Price $4.50.

For those interested in the history of bacteriology this volume will serve as a valuable reference source. It is a selected annotated bibliography divided into four parts. Part I lists abstracting and review periodicals, book reviews, catalogs, dictionaries, encyclopedias, etc., that deal with bacteriological subjects. Part II lists literature pertaining to specific areas of bacteriology. Under the headings, Plant Disease and Viruses, are found some of the books used as text and references by plant pathologists. Part III lists references which contain biographical information on bacteriologists. Part IV contains selected biographies of outstanding bacteriologists.

Fifty years of botany. 1958. Edited by WILLIAM CAMPBELL STEERE. McGraw Hill Book Co., New York. 638 pp. Price $10.00.

Published in honor of the fiftieth anniversary of the Botanical Society of America, this volume contains papers covering progress and outstanding achievements in the various fields of plant science. Thirty-seven of the forty papers appeared in Volumes 43 and 44 of the American Journal of Botany. Three papers are of particular interest to plant pathologists: "The fight with the fungi; or the rusts and rots that rob us, the blasts and blights that beset us," by James G. Horsfall; "Problems in preventing plant-disease epidemics," by E. C. Stakman; and "An anatomist's view of virus diseases," by Katherine Esau.

CHARTER MEMBERS AND PRESIDENTS OF APS

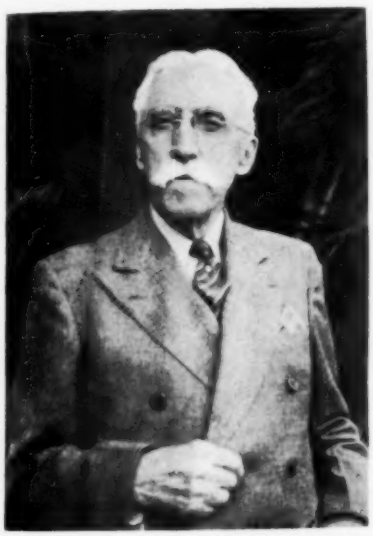
The 50th Anniversary Committee arranged an exhibit at the 1958 meetings of photographs of many prominent members of the Society. There have been numerous requests that at least the Charter Members and Presidents be published. Unfortunately, photographs of the following Charter Members were not available: Adelina Ames, S. M. Bain, O. M. Ball, W. S. Ballard, T. D. Beckwith, J. B. Dandeno, Anne Dorrance, Ethel Field, B. F. Floyd, G. T. French, Harrison Garman, J. C. Grossenbacher, J. G. Hall, H. A. Harding, W. O. Hart, Florence Hedges, R. P. Hibbard, C. J. Humphrey, C. N. Jensen, W. H. Lawrence, C. E. Lewis, W. Lochhead, R. E. B. McKenney, Frances Markham, G. F. Miles, W. J. Morse, P. J. O'Gara, Flora Patterson, J. B. Pollock, J. M. Reade, G. M. Reed, H. S. Reed, E. C. Rittue, J. L. Sheldon, C. E. Snyder, Alden Speare, M. B. Thomas, C. O. Townsend, Leva Walker, Errett Wallace, E. M. Wilcox, G. W. Wilson, Anna Wood.

Many persons provided photographs. Drs. J. E. Mitchell, E. W. Hanson, and R. W. Fulton compiled them. Eugene Herrling prepared them for publication. Receiving photographs from many sources makes possible errors in identification. We trust there are none.

Fiftieth Anniversary Committee

Wayne Bever
D. E. Ellis
J. G. Harrar
W. B. Hewitt
C. S. Holton
G. S. Pound, Chairman
H. A. Rodenhiser
J. R. Shay
R. H. Wellman

Charter Members



J.C. ARTHUR

H.W. BARRE

J.T. BARRETT

M.F. BARRUS

C.E. BESSEY

E.A. BESSEY

A. F. BLAKESLEE

H. L. BOLLEY

C. BROOKS

N. A. BROWN

A. H. R. BULLER

O. R. BUTLER

M. A. CARLTON

V. K. CHARLES

G. P. CLINTON

J. F. COLLINS

M. T. COOK

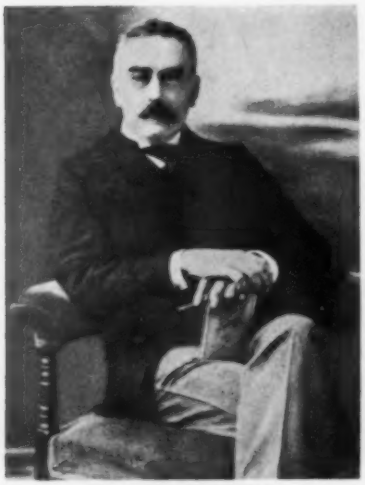
J. J. DAVIS

B. M. DUGGAR

J. W. EASTHAM

C. W. EDGERTON

H. A. EDSON



W. G. FARLOW

H. S. FAWCETT

H.M. FITZPATRICK

E.M. FREEMAN

H.R. FULTON

B.T. GALLOWAY

N.J. GIDDINGS

A.H. GILBERT

W.W. GILBERT

R.F. GRIGGS

H. GROH

H. T. GÜSSOW

B. D. HALSTEAD

R. A. HARPER

L. L. HARTER

C. HARTLEY

L. A. HAWKINS

F. D. HEALD

G. G. HEDGECOCK

W. T. HORNE

H. S. JACKSON

I. C. JAGGER

C. JAMIESON

E. C. JOHNSON

L. R. JONES

F. D. KERN

B. F. LUTMAN

L. McCULLOCH

T. F. MANNS

H. METCALF

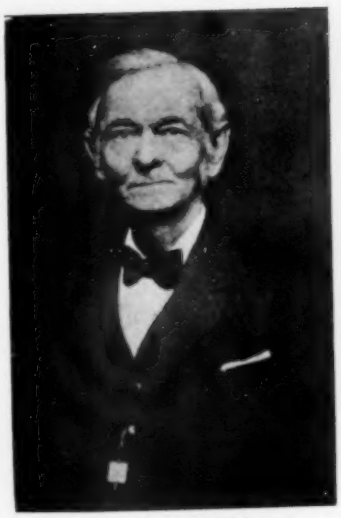
W. A. MURRILL

J. B. S. NORTON

E. W. OLIVE

W. A. ORTON

L. H. PAMMEL



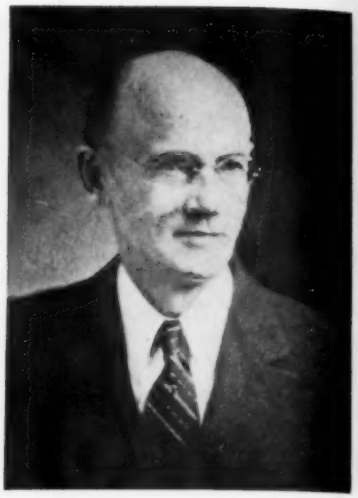
C. H. PECK

V. W. POOL

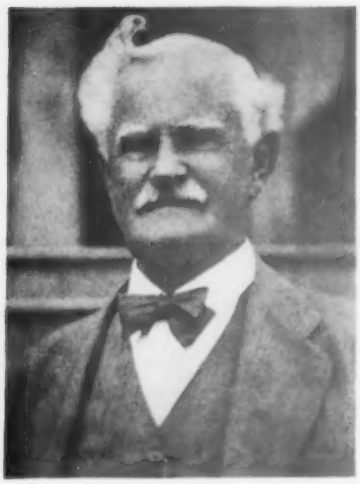
F. V. RAND

D. REDDICK

J.W. ROBERTS



F.M. ROLFS



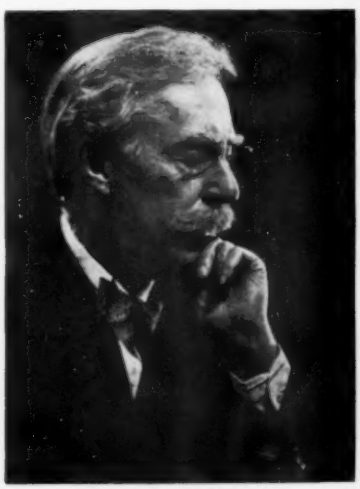
P.H. ROLFS

J.B. RORER

W.M. SCOTT

F.J. SEAVER

A.D. SELBY



H.B. SHAW

C.L. SHEAR

E.F. SMITH

R.E. SMITH

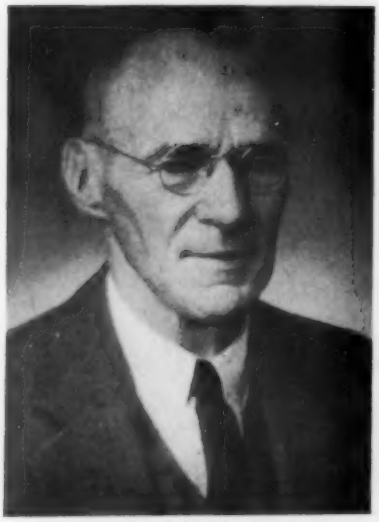
P. SPAULDING

F.L. STEVENS

F.C. STEWART

V.B. STEWART

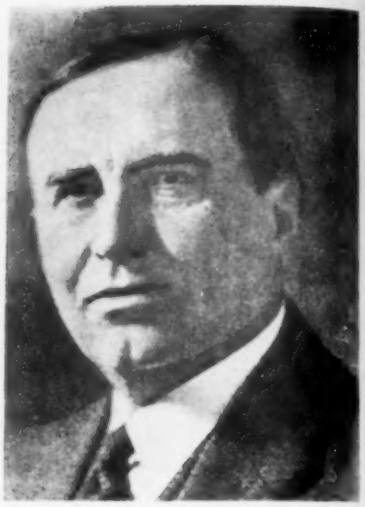
R.E. STONE



D.B. SWINGLE

J. J. TAUBENHAUS

R. THAXTER



J. M. VAN HOOK

H. VON SCHRENCK

M. B. WAITE

G. N. WALCOTT

H. H. WHETZEL

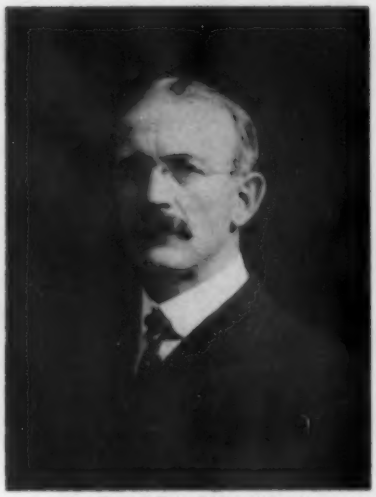
F. A. WOLF

A. F. WOODS

Presidents

L. R. JONES - 1909

F. L. STEVENS - 1910



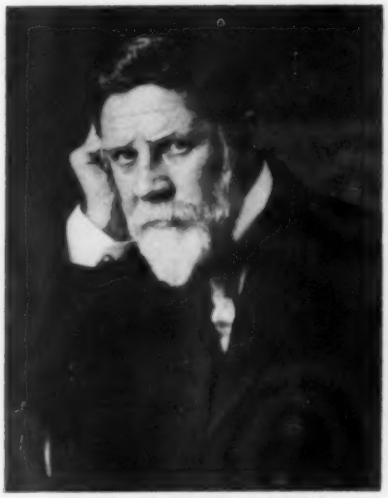
A. D. SELBY - 1911

G. P. CLINTON - 1912

F. C. STEWART - 1913

H. METCALF - 1914

H. H. WHETZEL - 1915



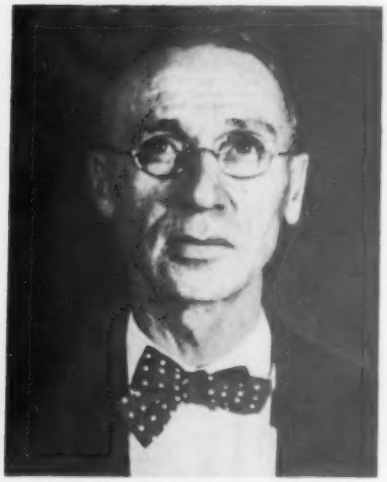
E. F. SMITH - 1916

M. T. COOK - 1917

E. M. FREEMAN–1918

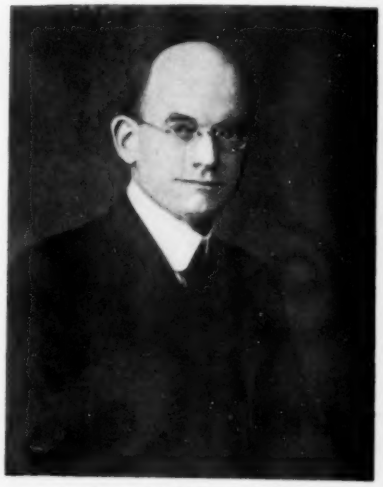
C. L. SHEAR–1919

W. A. ORTON–1920



D. REDDICK –1921

E. C. STAKMAN–1922



G. R. LYMAN–1923

F. D. FROMME–1924

C. W. EDGERTON–1925

I. E. MELHUS–1926

M.F. BARRUS – 1927

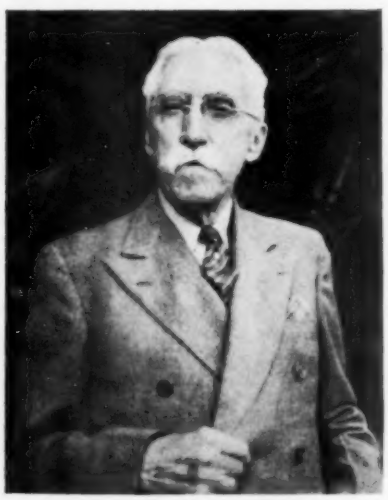
H.P. BARSS – 1928

R.J. HASKELL – 1929

H.S. FAWCETT – 1930

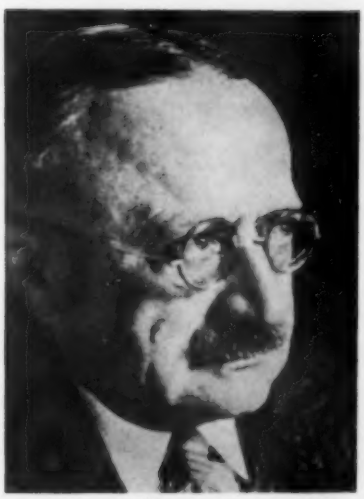
M.W. GARDNER – 1931

F.D. HEALD – 1932



J.C. ARTHUR – 1933

N.E. STEVENS – 1934

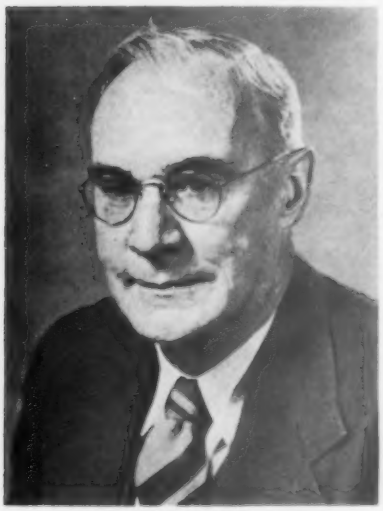


H.T. GÜSSOW – 1935

G. H. COONS – 1936

G. W. KEITT – 1937

H. W. ANDERSON – 1938



C. R. ORTON – 1939

C. CHUPP – 1940

J. G. LEACH – 1941

L. M. HUTCHINS – 1942

J. C. WALKER – 1943

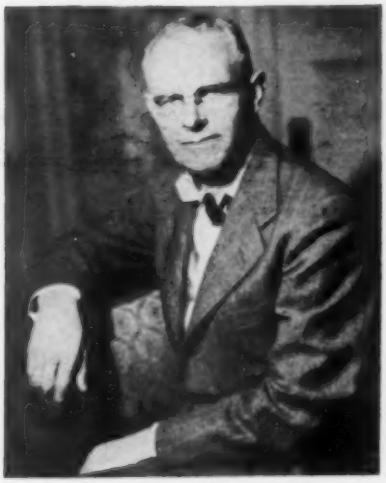
J. J. CHRISTENSEN – 1944

H. B. HUMPHREY – 1945

J. H. CRAIGIE – 1946

A. J. RIKER – 1947

R. S. KIRBY – 1948



W. D. VALLEAU – 1949

C. M. TUCKER – 1950

J. G. HORSFALL – 1951

G. L. McNEW – 1952

J. G. DICKSON – 1953

G. F. WEBER–1954

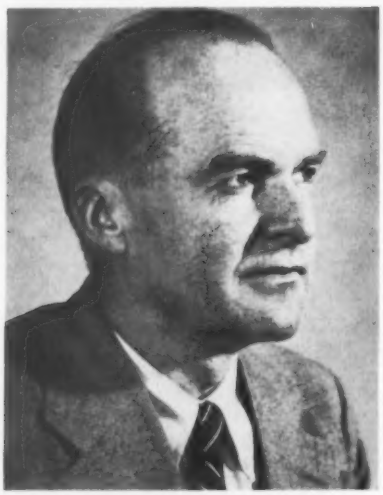
J. H. JENSEN–1955

H. HART–1956

G. W. FISCHER–1957

P. R. MILLER–1958

G. S. POUND–1959



W. C. SNYDER–1960